CHARACTERIZATION OF MOLECULAR AND GENETIC DETERMINANTS OF POLY (ADP-RIBOSE) POLYMERASE INHIBITOR SENSITIVITY IN OVARIAN CANCER

By

Pingping Fang

MS, Wuhan University, 2010

Submitted to the graduate degree program in Pharmacology and the Graduate Faculty of the University of Kansas Medical Center in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

_____

Co-chairperson: Wen-Xing Ding, PhD

_____

Co-chairperson: Jeremy Chien, PhD

_____

Jed Lampe, PhD

_____

Lisa (Yuxia) Zhang, PhD

_____

Andrew K. Godwin, PhD

Date Defended: March 12th, 2018

The dissertation committee for Pingping Fang certifies that this is the approved version of the following dissertation:

Characterization of Molecular and Genetic Determinants of

Poly (ADP-Ribose) Polymerase Inhibitor Sensitivity in Ovarian Cancer

_____

Co-chairperson: Wen-Xing Ding, PhD

_____

Co-Chairperson: Jeremy Chien, PhD

Date Approved: March 12th, 2018

Abstract

Ovarian cancer is the most leathal cancer among gynecologic cancers in the United States and is the fifth leading cause of cancer death among American women. Approximately sixty percent of women with ovarian cancer are diagnosed with advanced stage diseases. High-grade serous ovarian carcinoma (HGS-OvCa) is the most common subtype of epithelial ovarian cancer and accounts for around 70% of all ovarian cancers in the U.S. Although the platinum-taxane combination has been the standard of care for treatment of ovarian cancer for over 15 years, an emerging science indicates poly (ADP-ribose) polymerase inhibitors (PARPi) are also active in a substantial portion of HGS-OvCa. Cancer cells that show *"BRCAness"* are highly sensitive to PARPi, including those with deleterious mutations in *BRCA1/2* or defects in other components that are crucial for homologous recombination (HR) repair pathway. In HGS-OvCa, genes involved in HR are altered in about 50% of cases, making these cancers sensitive to PARPi. However, FOXM1 transcription factor network is activated in more than 84% of cases in HGS-OvCa, and activation of the FOXM1 pathway has been shown to upregulate genes involved in HR. Yet, the role of FOXM1 in PARPi response is not well studied. The *MYC* proto-oncogene is reported to be amplified in a significant number of epithelial ovarian cancer, and its role in the regulation of DNA repair and the PARPi response is not yet characterized. Therefore, we evaluated the roles of FOXM1 and MYC in PARPi response and explored ways to target them and develop new combinational therapies to overcome PARPi resistance. Besides, our understanding of PARPi resistance mechanism is not complete, and there is an urgent need to identify other potential regulators of PARPi response. So, we performed a genome-wide CRISPR knockout screen to identify novel regulators of PARPi response in ovarian cancer cells.

To identify potential regulators of PARPi response, we first evaluated the role of FOXM1 and found that PARPi olaparib induced the expression and nuclear localization of FOXM1, suggesting FOXM1 might play an important role in the adaptive response to PARPi. Using ChIP-qPCR, we observed that olaparib treatment enhanced the binding of FOXM1 to genes involved in HR. Using qRT-PCR, we found *FOXM1* knockdown by RNAi or treatment with thiostrepton, a natural peptide thiazole antibiotic, led to significantly decreased expression of FOXM1 and HR repair genes, such as *BRCA1, FANCF, BRCC3, BRIP1, NBS1* and *Csk1*. Consequently, with short term SRB assay and long-term colony formation assay, both *FOXM1* knockdown and thiostrepton treatment showed enhanced sensitivity to olaparib. Using comet and PARP trapping assays, we also observed increased DNA damage and PARP1 trapping in FOXM1-inhibited cells treated with olaparib. Finally, thiostrepton treatment lead to the decreased expression of *BRCA1* in rucaparib-resistant breast cancer cells and enhanced sensitivity to rucaparib. Collectively, these results suggest that FOXM1 plays an important role in the adaptive response induced by olaparib and that FOXM1 downregulation by siRNAs or thiostrepton treatment contributes to *"BRCAness"* and enhances sensitivity to PARP inhibitors.

Second, we found that *FOXM1* silencing results in c-MYC upregulation, and that *FOXM1* knockdown in itself is not sufficient to downregulate several HR genes, including *BRCA1, BRCA2, FANCF* and *BRIP1*. Analysis of the ENCODE (Encyclopedia of DNA Elements) database found that both FOXM1 and c-MYC bind to overlapping regions in the promoters of HR genes, such as *BRCA1* and *RAD51*, suggesting that these HR genes may be co-regulated by FOXM1 and c-MYC. In addition, using the Cancer Genome Atlas (TCGA) database we discovered that *FOXM1*, c-*MYC*, and *BRD4* are highly expressed in ovarian cancer and are related to each other, making them relevant therapeutic targets to explore. Using Western blot, we showed that BET bromodomain

inhibitor, (+)-JQ1, suppresses the expression of c-MYC and FOXM1 as well as BRCA1 and RAD51. By chromatin immunoprecipitation and quantitative polymerase chain reaction (ChIP-qPCR), we saw decreased binding of both FOXM1 and c-MYC to the promoters of *BRCA1* and *RAD51*, suggesting a direct regulation of these genes by FOXM1 and c-MYC. Treatment of three cancer cell lines (ES-2, OV90 and OVCA420*) with (+)-JQ1, lead to decreased cell viability in a dose-dependent manner for all cell lines. With SRB assay, we also observed mild to moderate synergistic effects of PARP inhibitors and (+)-JQ1 in cancer cells with primary resistance to olaparib as well as in cancer cells with acquired resistance to rucaparib. Strikingly, adding sub-lethal doses of (+)-JQ1 and olaparib completely inhibited colony formation. Taken together, these data suggest that inhibition of FOXM1 and c-MYC by BET inhibitor, (+)-JQ1, induces *"BRCAness"* by downregulating *BRCA1* and *RAD51*, and sensitizes resistant cancer cells to PARP inhibitors.

Third, using the sensitivity of PARPi olaparib as a surrogate, we performed a CRISPR/Cas9 based genome-scale loss-of-function screen and identified *C12orf5*, a gene that encodes a metabolic regulator, *TP53* induced glycolysis and apoptosis regulator (TIGAR) as a potential genetic determinant of PARPi response. To unveil the mechanisms involved in this process, further studies showed that *TIGAR* knockdown induces apoptosis, elevates the production of reactive oxygen species (ROS), and arrests cells at S-phase. These effects collectively contribute to accumulation of DNA damage and sensitize cancer cells to olaparib. More interestingly, RNA-sequencing analysis showed that *TIGAR* knockdown leads to decreased expression of *BRCA1*, and GSEA analysis indicates that *TIGAR* knockdown unexpectedly produces a gene signature that is similar to *BRCA1*-downregulated cells. Furthermore, *TIGAR* knockdown results in downregulation of genes associated with the Fanconi Anemia (FA) pathway. Collectively, these

findings provide us new insights into the molecular mechanisms involved in sensitization of olaparib after *TIGAR* knockdown. In addition to enhanced sensitivity to PARPi, *TIGAR* knockdown also inhibits cell growth by induction of cellular senescence as shown by SA-β-Gal staining analysis. Concomitantly, *TIGAR* knockdown resulted in the reduced efficacy in spheroid formation and the enhanced therapeutic effects of olaparib. Finally, analysis of TCGA database demonstrated that *TIGAR* amplification was found in different types of cancer including ovarian cancer (amplification was seen in about 11% of cases), and that elevated expression levels of *TIGAR* mRNA is associated with poor overall survival in high-grade serous ovarian cancer. Taken together, our data suggest that TIGAR negatively regulates PARPi sensitivity and is a relevant therapeutic target in ovarian cancer.

In summary, our studies showed that FOXM1 plays an important role in the adaptive response to PARPi and a potential role in the resistance to PARPi by positively regulating HR repair pathway through upregulation of *BRCA1, RAD51*, and other HR genes, such as FANCD2, FANCF, and RAD51D. Targeting FOXM1 with siRNA or treatment with thiostrepton induces a *"BRCAness"* phenotype and sensitizes cancer cells with either primary or acquired resistance to PARP inhibitors. The combination of FOXM1 inhibitor thiostrepton and PARPi enhances the cytotoxic effects and represents a new strategy to overcome PARPi resistance. We also showed that c-MYC works together with FOXM1 to regulate HR genes, including *BRCA1* and *RAD51*. Inhibition of FOXM1 and c-MYC by BET inhibitor downregulates HR genes, such as *BRCA1* and *RAD51*, and enhances cytotoxic effects of PARPi in resistant cells, suggesting that the combination of BET inhibitor with PARPi represents another effective strategy to induce "*BRCAness*" to overcome PARPi resistance in HR-proficient cancer cells. Lastly, we identified *TIGAR* as a novel regulator of PARPi response. Our data suggest that TIGAR negatively regulates PARPi sensitivity,

and *TIGAR* knockdown leads to increased cytotoxicity to olaparib by enhancing DNA damage, induction of apoptosis, and most importantly induction of *"BRCAness"* by downregulating *BRCA1* and the FA pathway. We also found that efficient *TIGAR* knockdown leads to cellular senescence. Collectively, TIGAR knockdown represents the third strategy to induce "*BRCAness*" to enhance sensitivity to PARPi. Considering that TIGAR knockdown also induces senescence and spheroid growth, TIGAR also serve as a target of monotherapy to induce dormancy in tumors to prevent cancer progression.

Acknowledgements

Thank you to my mentor Dr. Jeremy Chien, who I think is one of the most patient and supportive teachers that one can find. Your positive attitudes have always inspired me. You taught me to think critically and to be optimistic at the same time. You encouraged and guided me to be a better scientist. I have learned so much from you. It has been a wonderful journey to have you as a mentor.

Thank you to my thesis committee members, Dr. Wen-Xing Ding, Dr. Jed Lampe, Dr. Lisa Zhang and Dr. Andrew Godwin. Thank you for all your comments and suggestions that greatly facilitated in moving my research projects forward.

Thank you to the supporting staff for providing so nice and efficient coordination for graduate students. Thank you to all faculty members for their willingness to share their knowledge to teach us and give valuable feedbacks during seminars. Thank you to my peer graduate students in the department and in the IGPBS program, it has been a fun journey to study together with you all.

Thank you to all the Chien lab members, you are like families to me. Thank you to Kay Minn, who has helped a lot since the very first day I started rotation in the lab. Thank you for patiently fulfilling every request that I had, and for your friendship. Jill Madden, my best friend, thank you for always being so patient in listening to me and in helping me improve my English. I will always remember the fun times that we had together both in and outside the lab. Lisa Neums, thank you for helping me to get over with the toughest time when I was frustrated and depressed, for hanging out with me and my memories on those will never stop. Thank you to Megan Cooley, for sharing thoughts on research projects and personal life, thanks for your kindness, trust, and friendship, which I will cherish lifelong. Derek Oien, I enjoyed sitting next to you and you have

been an amazing neighbor, I enjoyed all the funny jokes that we had all the time, which I will miss so much, thank you for being a good listener and a good friend. Thank you to Cailin Wilson, who gave me lots of suggestions and encouragements, thanks for your help and friendship. Prabhakar Bastola, Cristabelle De Souza, Bernard Herrman, Stephan Graw, Richard Meier, Ying Wan, Yanchun Guo, Sunny (Lihua) Cheng, Dawson Foster, Katelin Gibson, and Lynn Magenheimer, Devin Santillan, Ashvini Vaidya, thanks for your support and friendship. I will always remember the Happy hours we had together.

I want to give my special thank you to my Mom and Dad. No matter what happens, you will always be there to back me up. Thank you for raising me up as a happy girl and for supporting me all the time. Thanks for your trust and for letting me become who I want to be. I am proud to be your daughter and my love to both of you will never end.

Thank you to my wonderful husband, Kaiwei, who always thinks I am a big fan of him. Indeed, I am the biggest fan of you and I grow prouder of you every day. I feel so lucky to have you being part of my life since I met you 10 year ago. You made my dream come true. You are always there whenever I need you. Thanks for supporting and encouraging me to be myself.

Dedicated

To my loving Mother & Father: Sukui Wang & Guibin Fang,

To my Grandmother: Chunying Li,

To my late Grandparents: Decheng Fang, Chenglian Wang, and Chengju Sun,

&

To my beloved husband: Kaiwei Liang

Table of Contents

List of Figures

List of Tables

List of Abbreviations

MYC	=	c-MYC proto-oncogene
%	=	Percent
11q13	=	Band q13 of chromosome 11
53BP1	=	Tp53-binding protein 1
ABC	=	ATP-binding cassette
ABCB1	=	P-glycoprotein 1
AKT	=	Protein kinase B
ANOVA	=	Analysis of variance
ATM	=	Ataxia-telangiectasia mutated
ATP	=	Adenosine triphosphate
ATR	=	Ataxia telangiectasia and Rad3-related
BCA assay	=	Bicinchoninic acid assay
BER	=	Base excision repair
BRAF	=	Serine/threonine-protein kinase B-raf
BRCA1/2	=	Breast cancer early onset 1/2
BRCC3	=	Lys-63-specific deubiquitinase BRCC36
BRIP1	=	BRCA1-interacting protein 1
C12orf5	=	Chromosome 12 open reading frame 5
Cas9	=	CRISPR associated protein 9
CCNE1	=	Cyclin E1
CDK	=	Cyclin-dependent kinase
CDK12	=	Cyclin-dependent kinase 12
ChIP	=	Chromatin immunoprecipitation
CHK1	=	Checkpoint kinase 1
CI	=	Combination index
CRISPR	=	Clustered Regularly Interspaced Short Palindromic Repeats
CtIP	=	C-terminus-binding protein of adenovirus E1A-interacting protein
CTNNB1	=	Beta-catenin
DMEM	=	Dulbecco's modified eagle medium
DMSO	=	Dimethyl sulfoxide
DNA	=	Deoxyribonucleic acid
DRR	=	DNA damage response
DSB	=	Double strand break
EGFR	=	Epidermal growth factor receptor
EMSY	=	BRCA2-interacting transcriptional repressor EMSY
ENCODE	=	Encyclopedia of DNA Elements
EOC	=	Epithelial ovarian cancer
F-2,6-BPase	=	Fructose-2,6-bisphosphatase

FA	=	Fanconi anemia
FANCA	=	Fanconi anemia complementation group A
FANCC	=	Fanconi anemia complementation group C
FANCD2	=	Fanconi anemia group D2 protein
FANCF	=	Fanconi anemia complementation group F
FBS	=	Fetal bovine serum
FDA	=	Food and drug administration
FDR	=	False discovery rate
FOXM1	=	Forkhead box protein M1
G1/2 phase	=	Gap 1/2 phase of the cell cycle
GAPDH	=	Glyceraldehyde 3-phosphate dehydrogenase
GeCKO	=	Genome-scale CRISPR knock-out
GSEA	=	Gene set enrichment analysis
γH2AX	=	H2AX phosphorylated at serine 139
H2DCFDA	=	2′,7′-Dichlorodihydrofluorescein diacetate
HBOC	=	Hereditary breast and ovarian cancer
HEK293T	=	Human embryonic kidney 293 cells with the SV40 T-antigen
HER2	=	Erb-b2 receptor tyrosine kinase 2
HGSOC	=	High-grade serous ovarian cancer
HR	=	Homologous recombination
hr	=	Hour
HRD	=	Homologous recombination defects
HRP	=	Horseradish peroxidase enzyme
HRR	=	Homologous recombination repair
HRT	=	Hormone replacement therapy
HSP90	=	Heat shock protein 90
IC_{50}	=	Half maximal inhibitory concentration
ICL	=	Interstrand crosslink
IGF1R	=	Insulin-like growth factor 1 receptor
IgG	=	Immunoglobulin G
KRAS	=	Gene encodes GTPase KRas
LIG3	=	DNA ligase 3
LMA	=	Low melting agarose
M phase	=	Mitotic phase of cell cycle
MAGeCK	=	Model-based analysis of genome-wide CRISPR/Cas9 knockout
MDR1	=	Multi-drug resistance 1
$MgCl_2$	=	Magnesium chloride
mL	=	Milliliter
mM	=	Milimolar
MMR	=	Mismatch repair

MOI	=	Multiplicity of infection
MRN	=	Complex consisting of Mre11, Rad50, and Nbs1
mt	=	Mutated
mTOR	=	Mechanistic target of rapamycin
MUC1-C	=	Mucin 1-C
NAC	=	N-acetyl-cysteine
NaCl	=	Sodium chloride
NAD+	=	Nicotinamide adenine dinucleotide
NADPH	=	Nicotinamide adenine dinucleotide phosphate
NBN/NBS1	=	Nibrin
NER	=	Nucleotide excision repair
NF-kB	=	Nuclear factor kappa-light-chain-enhancer of activated B cells
NH4Ac	=	Ammonium acetate
NHEJ	=	Non-homologous endjoining
nM	=	Nano molar
NTC	=	Non-targeting control
OC	=	Ovarian cancer
OMRF	=	Oklahoma medical research foundation
ORF	=	Open reading frame
P-gP	=	P-glycoprotein
pADPr	=	Poly(ADP-ribose)
PALB2	=	Partner and localizer of BRCA2
PARP	=	Poly(ADP-ribose) polymerase
PBS	=	Phosphate-buffered saline
PI	=	Propidium iodide
PI3K	=	Phosphoinositide 3-kinase
PIK3CA	=	Phosphoinositide 3-kinase catalytic subunit alpha isoform gene
PPP	=	Pentose phosphate pathway
PROTAC	=	Proteolysis targeting chimera
PTEN	=	Gene encodes phosphatase and tensin homolog
PVDF	=	Polyvinylidene fluoride
qRT-PCR	=	Quantitative reverse transcription polymerase chain reaction
RAD21	=	Double-strand-break repair protein rad21 homolog
RAD50	=	DNA repair protein RAD50
RAD51	=	DNA repair protein RAD51 homolog 1
RAD51C	=	DNA repair protein RAD51 homolog 3
RAD54	=	DNA Repair and recombination protein RAD54-like
RB	=	Gene encodes retinoblastoma protein
RFP	=	Red fluorescent protein
RNA	=	Ribonucleic acid

RNAi	=	RNA interference
RNase A	=	Ribonuclease A
ROS	=	Reactive oxygen species
RR	=	Rucaparib resistant
S phase	=	Synthesis phase of the cell cycle
S.D.	=	Standard deviation
SEM	=	Standard error of the mean
SA-β-gal	=	Senescence associated β -galactosidase
scr	=	Scrambled
SDS-PAGE	=	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
shRNA	=	Short hairpin RNA
siRNA	=	Small inference RNA
SLC25A40	=	Solute carrier family 25 member 40
SRB	=	Sulforhodamine B
SSB	=	Single strand break
TCGA	=	The cancer genome atlas
TIGAR	=	*TP53* induced glycolysis and apoptosis regulator
TP53	=	Tumor suppressor protein p53
Ve	=	Vehicle
VUS	=	Variant of unknown significance
wk	=	Week
WT	=	Wild- type
wt/vol	=	Ratio of weight over volume
X-gal	=	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside
µg/mL	=	Microgram per milliliter
µM	=	Micro molar

Chapter 1. Introduction

1.1 Background: Ovarian Cancer Facts and Figures

According to the SEER Cancer Statistics Review (CSR) 1975-2014, ovarian cancer is the most lethal gynecological cancer and the fifth leading cause of deaths among women in the United States (Howlader N 1975-2014). According to the same report, ovarian cancer is relatively rare, representing only 1.3% of all new cancer cases in the United States in 2017. In 2018, it is estimated that 22,240 women will be diagnosed with ovarian cancer and 14,070 deaths will result from complications associated with this disease. The lifetime risk of a woman to develop ovarian cancer is approximately 1 in 79 and to die from her disease is 1 in 108. Ovarian cancer is most frequently diagnosed among women aged between 55 and 64, and the median age at diagnosis is 63. The overall five-year survival rate for ovarian cancer is 46.5%. However, if diagnosed at early stages, the 5-year survival is up to 92.5% for women with stage I and 80% for stage II. The outcome decreases dramatically for advanced stage diseases, with a 5-year survival of 20-50% for women diagnosed with stage III and only 1-5% for those with stage IV. Unfortunately, only around 20% of patients are diagnosed at an early stage due to the lack of obvious symptoms. In general, death rates also increase with age, with the highest rate seen in women between 65 and 74 years of age. Although improved screening and advances in treatment are reducing the death rate by approximately 2.2% each year, concerted efforts are still needed to develop better screening to increase early diagnosis and to develop more effective treatments to improve survival of cancer patients.

Ovarian cancer was originally described as cancer that begins in ovaries (American Cancer Society 2017), which are the female reproductive glands. It was originally proposed to originate from one of the three cell types that constitute the ovaries: epithelial cells, stromal cells, or germ cells (Reid, Permuth et al. 2017). Epithelial ovarian cancer (EOC) accounts for more than 90% of

ovarian cancer, whereas stromal and germ cell-originated ovarian cancers represent 5-6% and 2-3%, respectively (Sankaranarayanan and Ferlay 2006). This dissertation focuses on EOC, which was originally proposed to originate from the surface epithelium of the ovary (Auersperg, Wong et al. 2001). However, emerging evidence suggest various subtypes of EOC have different tissues and cells of origin (Vaughan, Coward et al. 2011).

According to International Federation of Gynecology and Obstetrics (FIGO) staging system (Prat, Belhadj et al. 2015, Prat and Oncology 2015), there are four stages of EOC, namely stages I, II, III and IV. At stage I, ovarian cancer is confined to one or both ovaries. At stage II, cancer is regional, involving one or both ovaries and the uterus and/or fallopian tubes or other sites in the pelvis. Stage III is characterized by peritoneal metastases outside the pelvis whereas Stage IV is characterized by distant metastases, excluding peritoneal metastases.

EOC is a heterogeneous disease (Wang, Li et al. 2005). It can be histologically characterized into five main subtypes, which differ in their profiles of pathogenesis, cellular origin, genetic alterations, molecular changes and prognosis (Cancer Genome Atlas Research 2011, McCluggage 2011, Prat 2012, Song, Lee et al. 2013, Reid, Permuth et al. 2017). The five main subtypes are serous, mucinous, endometrioid, clear cell, and transitional (Brenner) cell (Chen, Ruiz et al. 2003). Based on distinctive morphologic and molecular genetic features, EOC is also classified into two different groups, type I and type II (Shih Ie and Kurman 2004, Kurman and Shih Ie 2010). Type I tumors usually have precursor lesions, often present at a low grade, and some are clinically indolent (Koshiyama, Matsumura et al. 2014). They include low-grade serous, low-grade endometrioid, clear cell, mucinous and transitional carcinomas. Type I tumors are commonly diagnosed at early stages and characterized by relatively stable genetic, lack of *TP53* mutations, and frequent mutations in *BRAF*, *KRAS*, *PTEN*, *CTNNB1,* and/or *PIK3CA* (Shih Ie and Kurman

2004). In contrast, type II carcinomas are more aggressive and highly genetically unstable with the majority possessing *TP53* mutations (Merajver, Pham et al. 1995, Salani, Kurman et al. 2008, Cho and Shih Ie 2009). Type II carcinomas are usually found at advanced stages and are composed of high-grade serous carcinoma, undifferentiated carcinoma, and malignant mixed mesodermal tumors. High-grade serous carcinoma (HGSC) makes up the majority of advanced-stage ovarian cancer cases and have the lowest survival rates (Dao, Schlappe et al. 2016). According to the Cancer Genome Atlas (TCGA) report, *TP53* is mutated in more than 95% of HGSCs (Cancer Genome Atlas Research 2011), and *BRCA1/2* mutations are found in approximately 25% of HGSCs. Mutations in other tumor suppressors, such as *CDK12, RB*, are reported in less than 10% of cases (Cancer Genome Atlas Research 2011).

For ovarian cancer, different treatment options are chosen depending on the histologic subtypes and the stages at diagnosis (Kim, Ueda et al. 2012, Trimbos 2017). Surgery, chemotherapy, and radiation are three basic forms of treatment for ovarian cancer. Surgery is still considered to be the main treatment for most ovarian cancers, with a goal to reduce the tumor burden to a nonvisible disease state. Moreover, the subtype and stage information are determined at the time of surgery, which includes removal of the ovaries and fallopian tubes, uterus, and portions of the omentum or other involved tissues which are feasible to remove (Shepherd 1989, Benedet, Bender et al. 2000, Weber, McCann et al. 2011). It is recommended that patients see a gynecologic oncologist for surgery, who has training and experience in treating, staging, and debulking ovarian cancer (Junor, Hole et al. 1999, Trope and Kaern 2006, du Bois, Rochon et al. 2009). Patients whose tumors have been optimally cytoreduced have a better outcome (Schorge, McCann et al. 2010) than those sub-optimally debulked patients. Women who have minimal

residual tumor after surgery have a median survival of 39 months versus 17 months for sub-optimally debulked individuals (Hoskins, McGuire et al. 1994, Bristow, Tomacruz et al. 2002).

The front-line chemotherapy for ovarian cancer patients is usually a combination of two or more drugs, which is given every 3-4 weeks. The standard systemic chemotherapy is the combination of a platinum agent, such as carboplatin or cisplatin, and a taxane agent, such as paclitaxel or docetaxel (Cannistra 2004). The use of carboplatin was shown to have less severe side effects than cisplatin while proven to be as effective (du Bois, Luck et al. 2003, Ozols, Bundy et al. 2003). Most often, the systemic chemotherapy for ovarian cancer can be administered by intravenous injection (IV) or intraperitoneal (IP) injection. Studies have shown that IP route has a better prognosis for progression-free survival and overall survival (Howell, Zimm et al. 1987, Armstrong, Bundy et al. 2006). However, due to complications, that included catheter infection, blockage, and leakage, this route of drug administration is not widely adopted as the standard of care. Standard chemotherapy is usually effective at initial treatment, and a pathologic or radiographic complete response is achievable in the majority of patients. However, the tumor relapse after several rounds of chemotherapy is common, and the acquired resistance eventually leads to treatment failure (Cannistra 2004, Chien, Kuang et al. 2013). A major issue associated with chemotherapeutic drugs in ovarian cancer is the severe side effects caused by damages to normal cells (Desoize and Madoulet 2002, du Bois, Luck et al. 2003, Gordon and Butler 2003, Ozols, Bundy et al. 2003, Shah and Dizon 2009). Some severe side effects, such as nausea and vomiting, loss of hair, hand and foot rashes, and mouth sores are temporary and dissipate once treatment is ceased. Chemotherapy can also cause damage to bone marrow (Florea and Busselberg 2011), leading to increased risk of infection, bleeding or bruising with minor cuts or injuries, and

fatigue. In addition, cisplatin has been shown to cause permanent kidney damage (Chu, Mantin et al. 1993, Meyer and Madias 1994).

Radiation therapy was used more often in the past to treat ovarian cancer (Arian-Schad, Kapp et al. 1990), and it is now rarely used as the first-line treatment for ovarian cancer (Lee, Park et al. 2011). It uses high energy x-rays or particles to kill cancer cells and also result in damage to surrounding normal cells. It can be useful in treating recurrent ovarian cancer that is refractory to chemotherapy (Lee, Park et al. 2011).

For patients with advanced-stage ovarian cancers, the current standard of care is surgical debulking followed by adjuvant chemotherapy to kill the residual cancer cells. In some cases, if the tumor burden is so extensive, neoadjuvant chemotherapy may be used to reduce tumor burden before the primary surgery. Although these chemotherapies are effective, they are not typically curative due to the development of acquired resistance. In the search for more effective treatments, targeted therapies have also been evaluated in patients with ovarian cancer. For example, bevacizumab, as a VEGF (Vascular Endothelial Growth Factor) inhibitor, has been tested in advanced EOCs and has been shown to shrink and slow tumor growth (Burger, Brady et al. 2011). These types of therapies in general were designed to be more specific in killing cancer cells while causing little damage to normal cells (Zhang, Yang et al. 2009). The first molecular targeted therapies were developed to target over-activated oncogenic proteins in cancer cells, such as Herceptin to target HER2-positive breast cancer (Slamon, Eiermann et al. 2011), vemurafenib to target the BRAF V600E mutants in melanoma (Tsai, Lee et al. 2008, Bollag, Hirth et al. 2010, Joseph, Pratilas et al. 2010, Chapman, Hauschild et al. 2011), imatinib mesylate to target BCR-ABL fusion proteins in leukemia (Druker, Tamura et al. 1996, Deininger, Goldman et al. 1997, Gambacorti-Passerini, le Coutre et al. 1997, Druker, Talpaz et al. 2001) and oncogenic

KIT/PDGFRA in gastrointestinal stromal tumor (van Oosterom, Judson et al. 2001, Demetri, von Mehren et al. 2002), and gefitinib to target the epidermal growth factor receptor (EGFR) tyrosine kinase in non-small cell lung cancer (NSCLC) (Lynch, Bell et al. 2004, Paez, Janne et al. 2004, Maemondo, Inoue et al. 2010). In high-grade serous ovarian cancer, copy number alteration (CNA) and genetic alterations in HR pathway are characteristic features of the disease (Cancer Genome Atlas Research 2011, Zack, Schumacher et al. 2013) and these features affect treatment options. Focal amplification of *CCNE1* was seen in ~20% of cases (Patch, Christie et al. 2015), and it was shown to be associated with primary treatment failure (Etemadmoghadam, deFazio et al. 2009) and reduced survival (Mayr, Kanitz et al. 2006). In addition, tumor suppressor genes (Cancer Genome Atlas Research 2011), such as *TP53*, *BRCA1/2*, *NF1*, *CDK12*, and *RB* are the most frequently mutated genes in high-grade serous ovarian cancer (Ahmed, Etemadmoghadam et al. 2010, Cancer Genome Atlas Research 2011). These mutations result in the loss of function, and gene therapies to restore the wild-type copy of mutated tumor suppressor genes have thus been unsuccessful.

Fortunately, recent advances provided a new approach to target the loss of function mutations associated with tumor suppressor genes. The functional loss associated with mutations in tumor suppressor genes may be targeted through synthetic lethal approach. Recently, PARP inhibitors have been developed to target *BRCA*-mutated cancer cells and have shown activity in a subset of ovarian cancers with a deficiency in homologous recombination repair (Vaughan, Coward et al. 2011). The mechanism of action associated with PARP inhibitors, such as olaparib (Lynparza), rucaparib (Rubraca), and niraparib (Zejula), and their FDA-approved indications for ovarian cancer patients will be discussed in the following sections.

1.2 Homologous recombination repair (HRR)

1.2.1 HRR is important in maintaining genome stability

Cancer is the disease of the genome, and loss of genome integrity is a hallmark of cancer (Hanahan and Weinberg 2011). Cells in our bodies are exposed to various sources of DNA-damaging agents, such as ultraviolet (UV), ionizing radiation, genotoxic chemicals, and reactive oxygen species (ROS) that are generated by cellular metabolism (Lindahl and Barnes 2000, Jackson and Bartek 2009). Exposure to these agents can lead to various types of DNA damages (Lindahl and Barnes 2000) and cells have evolved different and partially overlapping DNA repair mechanisms to repair these DNA damages (Hoeijmakers 2001). Briefly, nuclear excision repair (NER) repairs helix-distorting lesions that interfere with base pairing and generally obstruct transcription and normal replication (Marteijn, Lans et al. 2014). Base excision repair (BER) is used to fix single-strand breaks (SSBs) (David, O'Shea et al. 2007). Mismatch repair (MMR) is responsible for detection and repair of mispaired nucleotides generated from replication error (Kunkel and Erie 2015). DNA double-strand breaks (DSBs) are the most deleterious DNA damages, and if they are left unrepaired, it may lead to chromosomal rearrangement, genome instability, and finally cell death (van Gent, Hoeijmakers et al. 2001). In mammalian cells, DSBs are mainly repaired by HRR and non-homologous end joining (NHEJ) pathways (Pfeiffer, Goedecke et al. 2000). NHEJ is active throughout the cell cycle and predominantly used in the G_1 phase of cell cycle. It is error-prone because DSBs are fixed by simply ligating the breaks. This type of repair results in insertions, deletions, and translocations. In contrast, HRR is the most conservative and error-free repair mechanism for DSBs, and is preferentially used during S and G_2 phases of cell cycle, when the homologous templates on the sister chromatid are available (Paques and Haber 1999, Rothkamm, Kruger et al. 2003, Aylon, Liefshitz et al. 2004, Ira, Pellicioli et al. 2004, Moynahan and Jasin

2010). Therefore, HRR plays important roles in maintaining genome stability in mammalian cells. HRR is also the predominant mechanism for replication-associated DSBs repair that is activated when the replication machinery encounters SSBs or interstrand crosslinks (ICLs) (Arnaudeau, Lundin et al. 2001, Saintigny, Delacote et al. 2001). SSBs and ICLs produce stalled or collapsed replication forks when DNA polymerases encountered these sites. HRR plays an important role in the restart of the stalled or collapsed replication forks (Saleh-Gohari, Bryant et al. 2005). In mammalian cells, the important components involved in HRR pathways include Rad51, BRCA1/2, the Mre1/Ras50/Nbs1 complex, CtIP, and others. Mutations in any of these components have the potential to cause dysfunction in HRR, which can lead to reduced tolerance of DNA damage, and contribute to many human diseases, such as Fanconi Anemia, familial breast and ovarian cancer, and neurodegenerative diseases, suggesting that a faithful repair of DNA DSBs by HRR is essential for maintaining genome integrity, stability, and normal functions of the cell.

1.2.2 HRR process and its regulation

HRR-mediated DSB repair is a multi-step process (Jasin and Rothstein 2013). Firstly, DSBs are recognized by the Mre11/RAD50/Nbs1 (MRN) complex (Lee and Paull 2005), which serves to sense, stabilize and recruit other effector proteins (Figure 1.2.1). Then ataxia telangiectasia mutated (ATM), which is a serine-threonine kinase, phosphorylates the downstream effectors, such as H2AX, BRCA1, p53, 53BP1, Chk-2 and SMC1 (Shiloh 2003, Berkovich, Monnat et al. 2007). Secondly, 5' to 3' end resection to form 3'-end single stranded DNA (ssDNA) is mediated through multiple proteins and protein complexes (Symington and Gautier 2011), including MRN complex, C-terminal-binding protein interacting protein (CtIP), exonuclease 1 (EXO1), DNA 2 nuclease/helicase, Bloom syndrome protein (BLM), and other chromatin remodeling factors (Mimitou and Symington 2009, Liu and Huang 2016). Thirdly, RAD51 protein associates with

ssDNAs to facilitate the formation of nucleoprotein filament. During this process, RAD54, BRCA1, PABL2, and BRCA2 proteins participate in promoting RAD51 assembly at the ssDNA (Mazin, Alexeev et al. 2003, Sung and Klein 2006, Moynahan and Jasin 2010, O'Donovan and Livingston 2010, Holloman 2011). Fourthly, strand invasion after a search for homology region mediates the formation of D-loop, which is mediated by RAD51 and promoted by Mre11/Rad50/Xrs2 (MRX) complex. Next, an extension of the 3' end of the invading strand using homology template is achieved by several DNA polymerases but preferentially by DNA polymerase δ (Maloisel, Fabre et al. 2008). The 3' extensions by DNA polymerases produce double Holliday junctions that proceeds with branch migration, followed by DNA ligation by DNA ligase I. Finally, Holliday junctions are resolved by resolvase that separates the repaired DNA and the template (Figure 1.2.1).

HR pathway is extensively regulated, and it is dependent on the activity of various other pathways (Krajewska, Fehrmann et al. 2015), including DNA damage response, cell cycle regulation, protein stability control pathways, and growth factor-activated receptor signaling pathways. Components that regulate these related pathways will also regulate HR function. For example, Mre11/Rad50/Nbs1 (MRN) complex, ATM, ATR, CHK1 and others in DNA damage response pathway (Morrison, Sonoda et al. 2000, Sorensen, Hansen et al. 2005, Kozlov, Graham et al. 2011, Kocher, Rieckmann et al. 2012, Prevo, Fokas et al. 2012), cyclin-dependent kinases (CDKs) (*i.e.,* CDK2, 4, 6) in the cell cycle pathway (Aylon, Liefshitz et al. 2004, Branzei and Foiani 2008), heat-shock protein (HSP) family members (such as HSP90) and ubiquitination-related enzymes in the protein stability control pathway (Noguchi, Yu et al. 2006, Dungey, Caldecott et al. 2009, Zaidi, McLaughlin et al. 2012, Lafranchi, de Boer et al. 2014), and epidermal growth factor receptor (EGFR) pathway regulators (EGFR, HER2, IGF1R, etc.) in cell

proliferation pathway (Golding, Morgan et al. 2009, Mukherjee, McEllin et al. 2009, Myllynen, Rieckmann et al. 2011). However, the knowledge of how HR is regulated is thus far not complete. New knowledge and advances are being made using genetic screens with small interfering RNA (siRNA) or short hairpin RNA (shRNA) libraries to identify potential regulators of HR pathway in human cancer cells (Slabicki, Theis et al. 2010, Adamson, Smogorzewska et al. 2012).

Figure 1.2.1

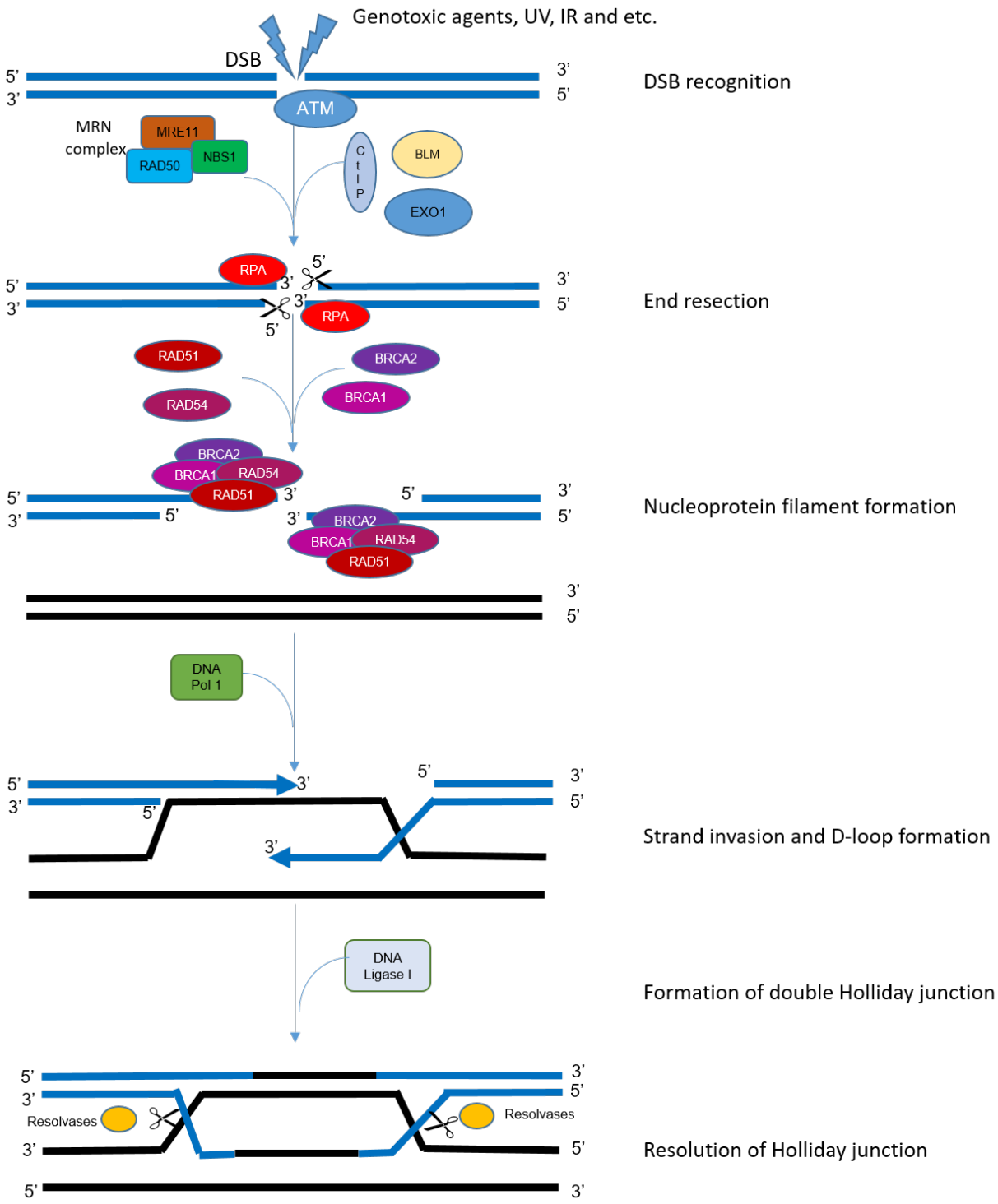

Genotoxic agents, UV, IR and etc.
DSB
5'
3'
3'
5'
ATM
MRN complex
MRE11
RAD50
NBS1
CtIP
BLM
EXO1
RPA
RAD51
RAD54
BRCA2
BRCA1
DNA Pol 1
DNA Ligase I
Resolvases
DSB recognition
End resection
Nucleoprotein filament formation
Strand invasion and D-loop formation
Formation of double Holliday junction
Resolution of Holliday junction

Figure 1.2.1: Schematic demonstration of HRR process. 1) DSB recognition; **2)** End resection; **3)** Formation of nucleoprotein filament; **4)** Strand invasion and D-loop formation; **5)** Formation of Double Holliday junctions; and **6)** Resolution of Holliday junction. DSB, double strand break; UV, ultraviolet; IR, ionizing radiation; ATM, serine/threonine kinase Ataxia-Telangiectasia mutated; BLM, Bloom Syndrome RecQ-like Helicase; EXO1, exonuclease I; RPA, single strand DNA binding protein Replication Protein A; DNA pol 1, DNA polymerase I.

1.2.3 Loss-of-function *BRCA1/2* mutations are associated with higher risks for ovarian and breast cancer

Breast cancer 1, early onset and breast cancer 2, early onset (BRCA1 and BRCA2) are tumor suppressors, which are encoded by *BRCA1* and *BRCA2* genes, respectively. They play important roles in DNA repair through HRR pathway (Silver and Livingston 2012). Inherited *BRCA1/2* mutations are associated with the majority of hereditary breast and ovarian cancer (HBOC), and are also associated with increased risk of other cancers and diseases, such as prostate cancer, pancreatic cancer, fallopian tube cancer, peritoneal cancer and Fanconi anemia (Brose, Rebbeck et al. 2002, Howlett, Taniguchi et al. 2002, Finch, Beiner et al. 2006, Levy-Lahad and Friedman 2007, Ferrone, Levine et al. 2009, Alter 2014, Sawyer, Tian et al. 2015). Together, *BRCA1* and *BRCA2* mutations account for 20-25% of hereditary breast cancers (Easton 1999) and approximately 15% of ovarian cancers (Pal, Permuth-Wey et al. 2005). A woman's lifetime risk of developing breast and/or ovarian cancer is greatly increased if she inherits a pathogenic mutation in *BRCA1* or *BRCA2.* About 12% of women in the general population will develop breast cancer sometime in their lives (Howlader N 2014). By contrast, 55-65% of women who inherited harmful *BRCA1* mutations and around 45% of women who inherited deleterious (disease-associated) *BRCA2* mutations will develop breast cancer by the age of 70 (Antoniou, Pharoah et al. 2003, Chen and Parmigiani 2007). About 1.3% of women in the general population will develop ovarian cancer sometime during their lives (Howlader N 2014). By contrast, 39% of women who inherited deleterious *BRCA1* mutations and 11-17% of women who inherited deleterious *BRCA2* mutations will develop ovarian cancer by the age of 70 (Antoniou, Pharoah et al. 2003, Chen and Parmigiani 2007). A recent study by Rebbeck and colleagues have described the characteristics of the 1,650 unique *BRCA1* and 1,731 unique *BRCA2* deleterious mutations identified in the Consortium of

Investigators of Modifiers of BRCA1/2 (CIMBA) database (Rebbeck, Friebel et al. 2018). However, not all *BRCA1/2* mutations are harmful or associated with increased risk of developing cancers. Moreover, in the clinic, there are some *BRCA1/2* mutations that are known as variants of unknown significance (VUS) because of not enough available clinical data to evaluate cancer risks associated with these mutations. The functional characterization of VUS in *BRCA1* and *BRCA2* is also an active area of research.

1.3 Poly (ADP-ribose) polymerase inhibitor (PARPi)

1.3.1 Synthetic lethality and therapeutic exploration of HR defects in the development of targeted therapy for cancer

The concept of synthetic lethality was first introduced in *Drosophila melanogaster* to describe the lethal relationship between two genes (Bridges 1922). As illustrated in Figure 1.3.1, synthetic lethality is defined as a type of genetic interaction in which the co-occurrence of two genetic events results in organismal or cellular death while the presence of only one genetic alteration will not (Hartman, Garvik et al. 2001, Boone, Bussey et al. 2007, Nijman 2011).

Figure 1.3.1

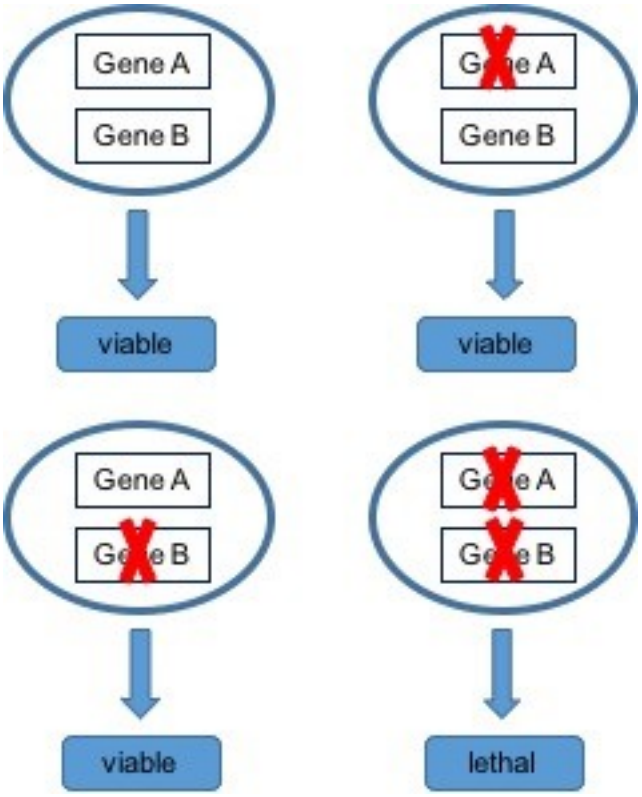


Figure 1.3.1: The concept of synthetic lethality. Two genes are synthetic lethal if the loss of expression of both leads to organismal or cell death while the loss of either of them is compatible with viability. Synthetic lethality also occurs between genes and small molecules, and can be used to elucidate the mechanism of action of drugs.

More recently, the concept of synthetic lethality has been extended to cancer cells, and the potential clinical utility of synthetic lethal-based targeted cancer therapy was best illustrated by two landmark studies by Bryant *et al.* (2005) and Farmer *et al*. (2005) (Bryant, Schultz et al. 2005, Farmer, McCabe et al. 2005). According to their studies, BRCA1 or BRCA2 dysfunction exquisitely sensitizes cancer cells to the inhibition of PARP1 enzymatic activity with PARP inhibitors, resulting in chromosomal instability, cell cycle arrest, and subsequent apoptosis of cancer cells. PARP1 inhibition specifically kills tumor cells while with minimal effects on normal cells (Bryant, Schultz et al. 2005, Farmer, McCabe et al. 2005). PARP1, poly (ADP-ribose) polymerase 1, is the founding member of a family of enzymes that exhibit homology in their active sites, where the dinucleotide NAD+ binds and is cleaved during mono- or poly- (ADP-ribosyl) action of protein substrates (Scott, Swisher et al. 2015). It is the best-understood enzyme among the three PARPs (PARP1, 2, 3) involved in BER-mediated SSB DNA repair (De Vos, Schreiber et al. 2012, Sousa, Matuo et al. 2012). At the sites of SSB, PARP1 binds to damaged DNA and undergoes a conformational change that realigns critical residues in the enzyme active site (Hassler and Ladurner 2012, Langelier, Planck et al. 2012, Langelier and Pascal 2013) to activate PARP1. After activation, PARP1 synthesizes poly-ADP-ribosylation (pADPr) chains to covalently bind to a variety of chromatin proteins, resulting in recruitment of additional DNA repair proteins to the damaged sites. In general, in the presence of PARP inhibitor, BER will be inactivated and this inhibition shifts the dependency on other essential DNA repair mechanisms, such as HRR, to fix DNA damages.

1.3.2 Mechanisms of PARP inhibitors

Several mechanisms were proposed to explain the synthetic lethality between *BRCA* mutations and PARP1 inhibition, and one mechanism is related to the inhibition of PARP enzymatic activity,

which is illustrated in Figure 1.3.2.A. In this model, unrepaired SSBs are converted to DSBs during DNA synthesis. DSBs are generally repaired by HRR during DNA replication. However, DSBs are not repaired by HRR due to HR defects caused by *BRCA* mutations. Unrepaired DSBs trigger other error-prone repair mechanisms, such as NHEJ, leading to genome instability and cell death.

Figure 1.3.2

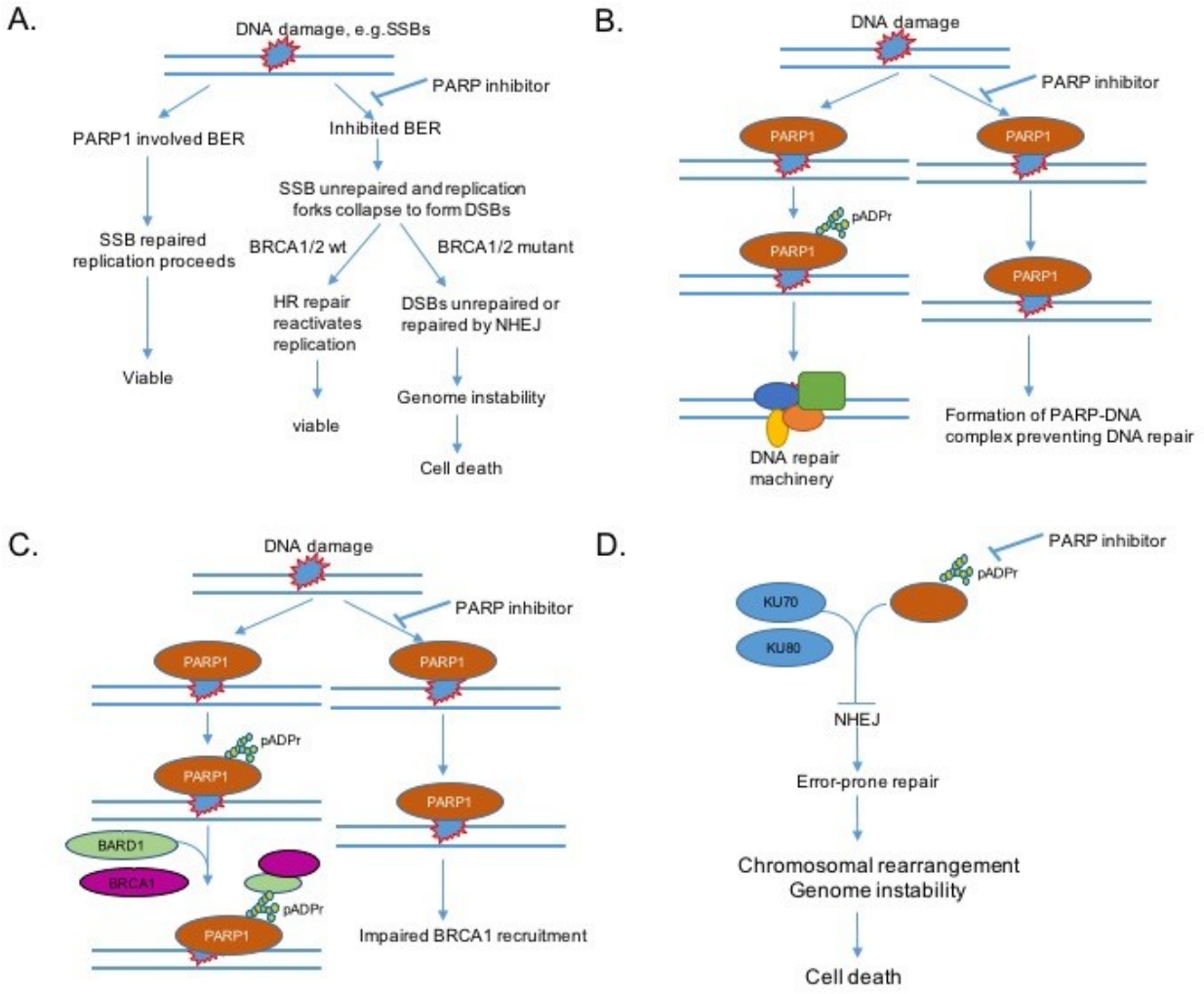


Figure 1.3.2: Mechanisms of PARP inhibitor. A) PARP inhibitor inhibits PARP1 enzymatic activity that is needed for BER to repair SSBs. PARP1 inhibition is synthetic lethal with *BRCA* mutations because unrepaired SSBs produce DSBs during DNA synthesis and these DSBs are not fixed by HRR due to HR deficiency caused by *BRCA* mutations. Unresolved DSBs induce cell death. **B)** PARP inhibitor leads to PARP1 trapping preventing DNA repair machinery recruitment to the damaged DNA. **C)** PARP inhibitor inhibits the pADPr formation and leads to impaired BARD1-mediated BRCA1 recruitment. **D)** PARP inhibitor reactivates NHEJ and promotes error-prone repair leading to genomic instability and cell death.

Another proposed mechanism (Figure 1.3.2.B) is that PARP1 trapping caused by PARP inhibitors at the damaged DNA inhibits the recruitment of BRCA1 and favors the NHEJ to promote genome instability (Scott, Swisher et al. 2015). PARP trapping by PARP inhibitors has gained increasing attention because studies show that PARP inhibitors that trap PARP proteins are more cytotoxic than genetic depletion of PARP (Horton, Stefanick et al. 2005, Heacock, Stefanick et al. 2010, Kedar, Stefanick et al. 2012, Murai, Huang et al. 2012). Moreover, the extent of SSB repair defects caused by PARP depletion is not as great as PARP inhibition by the inhibitors (Strom, Johansson et al. 2011). In the PARP trapping model, the resulting PARP-DNA complexes were considered to be more cytotoxic due to inhibition of recruitment of DNA repair machinery (Helleday 2011, Kedar, Stefanick et al. 2012). Murai *et al.* showed that the potency of PARP trapping is different among PARP inhibitors, and the one that is more potent to cause PARP trapping is more cytotoxic. Niraparib was shown to be more capable of PARP trapping than olaparib and veliparib, and it is the most cytotoxic among the three followed by olaparib and veliparib (Murai, Huang et al. 2012).

In addition, two other mechanisms have been proposed for PARP inhibitor (Scott, Swisher et al. 2015). One mechanism involves a deficiency in BRCA1 recruitment to damaged DNA (Figure 1.1.3.2.C). In the presence of PARP inhibitor, pADPr level decreases, leading to limited recruitment of pADPr-interacting protein BARD1, which is a BRCA1 binding partner, and the decrease in BARD1 recruitment to the damage sites results in a decrease in BRCA1 recruitment to the damaged DNA. In this case, the interaction between BARD1 and pADPr becomes especially critical for DNA repair process while *BRCA1* mutations impair the BRCA1/γH2AX interaction (Paull, Rogakou et al. 2000, Krum, la Rosa Dalugdugan et al. 2010), since both processes are needed for BRCA1 recruitment to the damaged DNA (Paull, Rogakou et al. 2000, Krum, la Rosa Dalugdugan et al. 2010, Li and Yu 2013). This potential mechanism can explain why cancer cells

haboring certain *BRCA1* mutations are hypersensitive to PARP1 inhibition. On the other hand, another mechanism suggests that the activation of NHEJ by PARP inhibitor in HR-deficient cells contributes to synthetic lethality between PARP inhibitor and HR defects (Patel, Sarkaria et al. 2011) (Figure 1.3.2.D). NHEJ components Ku70 and Ku80 were shown to interact with pADPr to suppress NHEJ (Hochegger, Dejsuphong et al. 2006, Wang, Wu et al. 2006, Paddock, Bauman et al. 2011), and this interaction will be reversed by a decrease of pADPr in the presence of a PARP inhibitor, thus leading to activation of NHEJ.

1.3.3 "*BRCAness*" and broader therapeutic scope for PARP inhibitors

Emerging evidence show that PARP inhibitor sensitivity is not restricted to *BRCA1/2* mutated cells. The term *"BRCAness"* was introduced to describe a phenomenon observed in some non-*BRCA* mutated sporadic cancers that display the clinical properties similar to hereditary *BRCA*-mutated cancers (Lord and Ashworth 2016). These cancers display HRR defects even in the absence of a germline *BRCA1* or *BRCA2* mutation. Several mechanisms have been identified to contribute to this *BRCA*-like phenotype, including genetic or epigenetic alterations in individual genes in HRR pathway and the Fanconi anemia pathway (Turner, Tutt et al. 2004). In high-grade serous ovarian cancer, around 50% of cases display HR deficiency (Cancer Genome Atlas Research 2011), and both germline and somatic *BRCA1/2* mutations only account for around 22% of ovarian cancers, indicating that there are other factors affecting HR function besides *BRCA1/2* mutations. The depletion of HR repair components other than BRCA1/2 (*i.e.,* the MRN complex, PALB2, RAD51, RAD54, NBS1, ATR, ATM, CHK1/2, FANCD2, FANCA and FANCC) shows synthetic lethality with PARP inhibition (McCabe, Turner et al. 2006, Dedes, Wilkerson et al. 2011, Lupo and Trusolino 2014). In addition, the amplification of *EMSY* gene, which encodes a

BRCA2-interacting protein that suppresses *BRCA2* transcription, also leads to *"BRCAness"* (Hughes-Davies, Huntsman et al. 2003). Finally, alterations in other non-HR genes were also shown to confer PARP inhibitor sensitivity, such as transmembrane protease serine 2 *(TMPRSS2)-ERG* translocation in prostate cancer (Brenner, Ateeq et al. 2011) and *EWS-FL11* or *EWS-ERG* (Brenner, Feng et al. 2012) translocations in Ewing sarcoma. These findings suggest that *"BRCAness"* resulted from direct or indirect alterations of HR components and other non-HR components, leads to sensitivity to PARP inhibitor and PARP inhibitor have a broader therapeutic scope beyond *BRCA* dysfunction.

1.3.4 PARP inhibitors in the clinic

The landmark pre-clinical studies have led to the rapid clinical development of PARP inhibitors as targeted therapy for *BRCA*-mutated cancers (Audeh, Carmichael et al. 2010, Fong, Yap et al. 2010, Tutt, Robson et al. 2010). Olaparib was one of the first PARP inhibitors that have been tested in clinical trials as a single agent in heavily pre-treated breast cancers and advanced ovarian cancers with the *BRCA1* or *BRCA2* mutations (Fong, Boss et al. 2009, Audeh, Carmichael et al. 2010, Tutt, Robson et al. 2010, Ledermann, Harter et al. 2012, Ledermann, Harter et al. 2014). It showed an overall response rate of around 41% and 33% in the breast and ovarian cancers respectively with a very tolerable side effect profile (Audeh, Carmichael et al. 2010, Fong, Yap et al. 2010, Tutt, Robson et al. 2010). The common side effects that occur in greater than 30% of patients taking olaparib are nausea, vomiting, diarrhea, abdominal pain, decreased hemoglobin, fatigue, decreased white blood cell count, anemia and increased serum creatinine (Fong, Boss et al. 2009). Olaparib has shown the most encouraging efficacy in platinum-sensitive high-grade serous ovarian cancer (Scott, Swisher et al. 2015), and in December 2014, United States Food and Drug Administration (FDA) approved it as a monotherapy to treat patients with advanced stage

ovarian cancer, who have germline *BRCA1/2* mutations and have been through three or more rounds of chemotherapies.

At present, two additional PARP inhibitors, rucaparib and niraparib, have been approved by FDA for ovarian cancer. In December 2016, rucaparib was granted accelerated approval for treatment of advanced ovarian cancer patients with deleterious *BRCA* mutation (germline and/or somatic), who have been treated with two or more lines of chemotherapies. This approval was based on data from two multicenter, single-arm, open-label clinical trials (Kristeleit, Shapira-Frommer et al. 2016, Oza, Tinker et al. 2017), in which efficacy of rucaparib was evaluated in 106 patients with advanced ovarian cancer, who had cancer progression after treatment with two or more rounds of chemotherapies. The clinical trials showed an investigator-assessed objective response rate (ORR) of 54% (57/106). The median duration of response (DoR) for the responders was 9.2 months. Most interestingly, the investigator-assessed ORR in platinum-sensitive patients was 66%, compared to only 25% in platinum-resistant and 0% in refractory patients, suggesting a preferential response in platinum-sensitive cohort.

Niraparib was approved by FDA in March 2017 to be used as a maintenance therapy for adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer, who are in complete or partial response to platinum-based chemotherapy. The approval was based on results from a randomized trial (NOVA) of 553 patients (Mirza, Monk et al. 2016), who were divided into the germline *BRCA*-mutated (g*BRCA*mut) and non-g*BRCA*mut cohorts. Overall, patients with niraparib had a significantly longer median progression-free survival (PFS) than those with placebo. Not surprisingly, the g*BRCA*mut cohort showed the biggest difference, with 21 months (niraparib) vs. 5.5 months (placebo). In addition, the preferential benefit was also seen with patients having tumors that showed HRD in the non- g*BRCA*mut cohort. In non-g*BRCA*mut

patients, the estimated PFS for patients taking niraparib was 9.3 months while only 3.9 months for those taking placebo.

Not only in ovarian cancer, PARP inhibitor also showed clinical activity in breast cancer. In Jan 2018, FDA extended the approved use of olaparib to treat patients with metastasized breast cancer and with a g*BRCA*mut. This is the first PARP inhibitor that has been approved to treat cancer other than ovarian cancer. Presently, PARP inhibitors are actively tested in various clinical trials as mono- or combinational-therapy in different types of cancer, including advanced hematological malignancies, triple-negative breast cancer, NSCLC, prostate cancer, colorectal cancer and metastatic melanoma (Scott, Swisher et al. 2015).

1.3.5 PARP inhibitor resistance

Although PARP inhibitors have shown considerable promises, drug resistance is inevitable and it will become a substantial clinical issue in the near future (Lord and Ashworth 2013). Like many other cancer drugs, development of resistance is expected to be a major contributor to treatment failure with PARP inhibitors. PARP inhibitor resistance can be intrinsic, with no response from the very beginning of treatment, or acquired, which develops after the initial response. Several mechanisms have been identified for PARP inhibitor resistance, some of which have also been validated in clinic (Lord and Ashworth 2013, Bouwman and Jonkers 2014). Overall, these mechanisms can be characterized into two main categories: (1) restoration of normal HR function, such as *BRCA* revertant mutation, 53BP1 loss and *EMSY* downregulation; (2) other non-HR related mechanisms, such as upregulation of efflux transporters, ribosomal protein S6, NF-kB signaling, and *CCNE1* amplification.

(I) Restoration of HR proficiency confers PARP inhibitor resistance

As PARP inhibitor sensitivity is largely dependent on HR defects, it is not surprising that restoration of HR function is the most common mechanism of PARP inhibitor resistance. HR function restoration in tumor cells can be achieved by different mechanisms, including direct changes in the HR components, such as secondary mutations of *BRCA* genes (McCabe, Turner et al. 2006, Sakai, Swisher et al. 2008), and alterations of other non-HR components, such as loss of 53BP1 and lack of *EMSY* amplification.

Resistance through secondary mutations of BRCA genes

Reversion mutations of *BRCA1* or *BRCA2* that resume the open reading frames (ORFs) to produce functional BRCA proteins is the most frequently observed mechanism to restore HR functionality in PARP inhibitor resistant tumors. The initial experimental evidence was provided by two independent studies (Edwards, Brough et al. 2008, Sakai, Swisher et al. 2008), showing that secondary mutations in mutated *BRCA2* restored its ORF to produce functional BRCA2 and regained HR function, leading to drug resistance. Later on, this preclinical mechanism was validated in tumor samples from a patient who did not respond to chemotherapy (Norquist, Wurz et al. 2011). Even though direct evidence of *BRCA1* reversion mutation leads to PARP inhibitor resistance is lacking, restored ORF of *BRCA1* is reported in the acquired resistance to cisplatin in *BRCA1*-mutated tumor samples (Norquist, Wurz et al. 2011). This reversion mutation mechanism may also be applicable to the acquired resistance to PARP inhibitor according to the BRCA/PARP functional dependency as suggested by B. Lupo, *et al*. (Ashworth 2008, Lupo and Trusolino 2014).

Resistance through loss of 53BP1

The restoration of a balance between HR and NHEJ activity is another mechanism that confers PARP inhibitor resistance. Loss of 53BP1, Tp53-binding protein 1, belongs to this category. PARP inhibitor cytotoxicity in HR-deficient cells is partly contributed by stimulation of the error-prone NHEJ that enhances genome instability (Patel, Sarkaria et al. 2011). These results suggest that the NHEJ-dominant repair underlies PARP inhibitor sensitivity. Several studies have shown that 53BP1 loss can restore the balance between HR and NHEJ activities and confer PARP inhibitor resistance in *BRCA1*-mutated cancer cells (Cao, Xu et al. 2009, Bouwman, Aly et al. 2010, Bunting, Callen et al. 2012, Jaspers, Kersbergen et al. 2013). 53BP1 deficiency promotes the processing of DSB DNA ends to produce single-stranded DNA, which reestablishes the use of HR pathway to repair DSBs (Lord and Ashworth 2013).

Resistance through stabilization of mutant BRCA1

Some mutations in *BRCA1* produce less stable, misfolded and partially functional BRCA1 proteins, thereby causing the loss-of-function effect. In tumor cells with these mutants, increasing the stability of mutant BRCA1 could restore HR function and lead to PARP inhibitor resistance. For example, HSP90 (heat shock protein 90) is a chaperone protein that assists in the folding of nascent proteins and unfolded proteins upon heat stress. HSP90 also assists in the folding of misfolded proteins caused by mutations and provides a phenotypic stability (Rutherford and Lindquist 1998, Queitsch, Sangster et al. 2002). It also stabilizes a number of proteins required for tumor growth (Richter and Buchner 2001), including mutant BRCA1. Johnson *et al.* showed that HSP90 contributes to PARP inhibitor (Rucaparib) resistance by stabilization of mutant BRCA1, which has a mutation in the BRCT domain affecting protein folding but retains a residual activity. Through HSP90-mediated stabilization, cells with this *BRCA1* mutation regained HR function,

which can be reversed using HSP90 inhibitors, indicating the direct involvement of HSP90 in conferring PARP inhibitor resistance (Johnson, Johnson et al. 2013).

Other possible mechanisms to restore HR function

Besides the mechanisms discussed above, additional mechanisms may regulate HR and contribute to PARP inhibitor resistance. One mechanism is through the regulation of *EMSY* gene expression. Hughes-Davies *et al.* showed that *EMSY* gene encodes a BRCA2-associated protein (EMSY), which is frequently amplified in sporadic breast and ovarian cancers (Hughes-Davies, Huntsman et al. 2003). It is also considered as a key oncogene within the 11q13 amplicon in ovarian cancer (Brown, Irving et al. 2006). Increased level of EMSY was suggested to repress BRCA2 transactivation and induce chromosomal instability, mimicking the effect of *BRCA2* mutation in the development of hereditary breast/ovarian cancer through disrupting BRCA2/RAD51 pathway in the DNA-damage response (Cousineau and Belmaaza 2011). Therefore, downregulation of EMSY would reverse the effects described above and serve as a possible mechanism for PARP inhibitor resistance.

(II) Non-HR related mechanisms

Increase expression of drug efflux transporters

PARP inhibitor response can be affected by ATP-binding cassette (ABC) transporters, which are transmembrane proteins that carry substrates, including many drugs, across cell membranes (Lord and Ashworth 2013). ABC transporters have been implicated in multidrug resistance (Bellamy 1996). One example is P-glycoprotein efflux pump (also known as PgP, MDR1, and ABCB1), overexpression of which has been reported to reduce the efficacy of numerous drugs by increasing their efflux from the cells (Shen, Fojo et al. 1986, Fojo, Ueda et al. 1987, Parekh, Wiesen et al.

1997, Choi and Yu 2014, Cooley, Fang et al. 2015). This is also a recognized molecular mechanism for treatment failure in cancer patients. Several studies have shown that PgP expression in cancer cells contributes to chemotherapy and PARP inhibitor resistance, which can be reversed by PgP inhibitors (Wurzer, Herceg et al. 2000, Dumitriu, Voll et al. 2004, Rottenberg, Jaspers et al. 2008, Lawlor, Martin et al. 2014). It is suggested by Lupo *et al.* (Lupo and Trusolino 2014) that inhibition of PARP would enhance efflux pump expression by various mechanisms. In addition, Patch *et al.* identified a new mechanism involving promoter sequence rearrangement between *SLC25A40* and *ABCB1* (Patch, Christie et al. 2015), which results in the high expression of *ABCB1*. This promoter rearrangement was found in tumor samples from patients with chemotherapy-resistant high-grade serous ovarian cancer. Olaparib was suggested to be a substrate of MDR1 (Rottenberg, Jaspers et al. 2008, Lawlor, Martin et al. 2014, Vaidyanathan, Sawers et al. 2016) and direct evidence is needed to support that MDR1 upregulation also contributes to resistance to other PARP inhibitors. In this regard, development of PARP inhibitors that are not substrates for MDR1 might decrease the possibility of PARPi resistance caused by MDR1 upregulation.

Decreased PARP expression and activity

A well-recognized mechanism of antibiotics resistance in bacteria is loss of their cellular targets (Dever and Dermody 1991). Likewise, this can be a potential mechanism for PARP inhibitor resistance. A study showed that cells with loss-of-function PARP1 mutants are 100-fold more resistant to olaparib than wild-type cells (Murai, Huang et al. 2012), and cells with low levels of PARP1 are less responsiveness to PARP inhibitors (Farmer, McCabe et al. 2005). In addition, human cancer cells that have acquired resistance to PARP inhibitor, veliparib, have lower expression of PARP1 (Liu, Han et al. 2009). These findings support the idea that loss of PARP1 is one of the mechanism contributes to PARP inhibitor resistance.

Increased phosphorylation of ribosomal protein S6

Studies conducted by Sun and colleagues (Sun, Zhang et al. 2014) suggest that activation of BRCA1-PI3K/Akt/mTOR axis contributes to acquired resistance to PARP inhibition, which might result from the enhanced DNA repair through the phosphorylation of ribosomal protein S6 (Khalaileh, Dreazen et al. 2013). They showed that prolonged PARP inhibitor exposure leads to an increase in phosphorylation of ribosomal protein S6 in BRCA1-deficient cells but not in BRCA1-proficient cells, which is further validated by the observation that BRCA1-deficient cells with a mutation that impairs phosphorylation of ribosomal protein S6 did not develop PARP inhibitor resistance even after long-term exposure (Sun, Zhang et al. 2014). These findings suggest that the increased phosphorylation of ribosomal protein S6 represents a component of a signaling pathway that potentially contributes to PARP inhibitor resistance.

Up-regulation of NF-kB signaling pathway

NF-kB is a transcription factor and is often up-regulated in many types of cancers, leading to activation of cancer cell proliferation and migration as well as a decrease in apoptosis (Karin 2006). By comparing the gene expression between parental and PARP inhibitor-resistant UWB1.289 cells, Nakagawa *et al.* showed that upregulation of NF-kB signaling is involved in PARP inhibitor resistance. Pharmacologically inhibition of NF-kB pathway and downregulation of its key components re-sensitize resistant cells to PARP inhibitors (Nakagawa, Sedukhina et al. 2015), suggesting that NF-κB pathway plays a role in PARP inhibitor resistance.

Amplification of CCNE1 gene

Besides the mechanisms mentioned above, new mechanisms of chemotherapy resistance are unveiled using next-generation sequencing analysis of tumor samples from patients. Patch *et al.*

conducted a whole-genome sequencing of tumors and germline DNA samples from 92 patients with refractory disease, primary resistance to chemotherapy, or matched pairs of sensitive and acquired resistant high-grade serous ovarian cancers (Patch, Christie et al. 2015). They found that *CCNE1* gain/amplification was a common event in both primary resistant and refractory diseases (19% cases), and according to their clinical association analysis, the amplification of chromosome region involving *CCNE1* was also the dominant structural variant associated with primary treatment failure. Interestingly, *CCNE1* gain/amplification and BRCA1/2 pathway disruption are mutually exclusive. Genome-wide shRNA synthetic lethal screen indicates that BRCA1 is selectively required in cancers that harbor *CCNE1* amplification (Etemadmoghadam, Weir et al. 2013), confirming the mutual exclusivity of *CCNE1* amplification and BRCA1/2 loss in high grade serous ovarian cancer. In cancer cells with *CCNE1* amplification where BRCA or HR function is selectively retained, these cells are expected to be resistant to PARP inhibitors because BRCA or HR deficiency is a requirement for PARP inhibitor sensitivity in cancer cells (Bryant, Schultz et al. 2005, Farmer, McCabe et al. 2005).

1.3.6 Enhancing therapeutic effects of PARP inhibitor

In order to achieve a durable response to PARP inhibitor and expand the clinical indication of PARP inhibitors to benefit more cancer patients, it is urgent to find ways to overcome resistance and enhance their therapeutic effects. Combination therapies are effective strategies to decrease resistance and achieve better therapeutic effects of chemotherapies (Baselga, Cortes et al. 2012, Yap, Omlin et al. 2013, Quinn, Dash et al. 2015, Bayat Mokhtari, Homayouni et al. 2017). Similarly, combination therapies can be used to enhance PARP inhibitor therapeutic effects. Because PARP inhibition impairs the cells' ability to respond to DNA damage, many groups have tested PARP inhibitors in combination with standard cytotoxic chemotherapy agents to enhance

the cell killing (Rodon, Iniesta et al. 2009), such as in combination with platinum in ovarian cancer and breast cancer (Burgess and Puhalla 2014), and in combination with temozolomide in breast cancer, glioblastoma, melanoma and acute leukemia (Palma, Wang et al. 2009, Barazzuol, Jena et al. 2013, Murai, Zhang et al. 2014). PARP inhibitor was also used together with signal transduction inhibitors, for example, gefitinib, in *EGFR*-mutated non-small-cell lung cancer (Burgess and Puhalla 2014, O'Sullivan, Moon et al. 2014). However, the optimal combination of PARP inhibitor and other chemotherapy drugs remains to be established. The optimal dose, sequence, and treatment frequency of PARP inhibitor and chemotherapeutic drugs need to be determined to maximize efficacy while minimizing toxicity (Yap, Sandhu et al. 2011). For example, in Phase I and Phase II studies, olaparib in combination with carboplatin (Lee, Hays et al. 2014) or cediranib (Liu, Barry et al. 2014) has shown efficacy against ovarian cancer. However, hematologic toxicity prevented continuous dosing of olaparib when combined with typical carboplatin doses (Lee, Hays et al. 2014).

The mechanisms underlying the potential synergies of drug combinations can be attributed to the following: First, in cells with functional HR or Fanconi anemia (FA) pathway, the other agent suppresses the expression of HR repair genes and induce HRD, resulting in PARP inhibitor hypersensitivity. Various studies have shown that HRD can be induced by a variety of agents. For example, phosphatidylinositol 3-kinase (PI3K) inhibitors downregulate *RAD51* or *BRCA1/2* in breast cancer (Ibrahim, Garcia-Garcia et al. 2012, Juvekar, Burga et al. 2012), epidermal growth factor receptor (EGFR) inhibitors promote BRCA1 trafficking from the nucleus to the cytoplasm in triple-negative breast cancer (Nowsheen, Cooper et al. 2012), and ATR inhibitors diminish replication stress-induced activation of cell-cycle checkpoints and repair in ovarian cancer cells (Huntoon, Flatten et al. 2013). Second, these agents could interfere with other roles of PARP1

(Scott, Swisher et al. 2015). Besides its role in BER, PARP1 also participates in HR and NHEJ, and activation of NHEJ pathway after PARP inhibition is one of the potential mechanism contributing to cytotoxic effects of PARP inhibitor in cancer cells (Patel, Sarkaria et al. 2011). Accordingly, loss of 53BP1 suppresses NHEJ and restore the balance between HR and NHEJ repair, leading to PARP inhibitor resistance (Bunting, Callen et al. 2010). These studies suggest that strategies to activate NHEJ and inhibit HR at the same time may serve as an option to enhance PARP inhibitor sensitivity (Scott, Swisher et al. 2015).

Meanwhile, a better understanding of the PARP inhibitor resistance mechanisms would provide us additional options to develop more effective combinational therapies to overcome the resistance. The genome-wide, loss-of-function screen is an important tool to identify novel HR modifiers and to expand the growing list of potential therapeutic targets that control HR. These HR modifiers could serve as novel targets to enhance PARPi sensitivity. Genome-wide siRNA screen is the approach that has been used by several groups to find new modifers of DNA repair pathways (Lord, McDonald et al. 2008, Slabicki, Theis et al. 2010, Adamson, Smogorzewska et al. 2012). These studies identified candidate modifiers of PARP inhibitor sensitivity, and these genes are involved in DNA response and repair pathways as well as DNA replication (Lord, McDonald et al. 2008, Bajrami, Frankum et al. 2014). In addition, components of proteasome or RNA biology were identified as novel HR modifiers (Krajewska, Fehrmann et al. 2015). These screens have greatly enhanced our understanding of human DNA repair processes and led to the discovery of a number of novel genes implicated in various aspects of DNA repair. In chapter 4, we used a genome-scale CRISPR/Cas9 knockout pooled library (GeCKO library) to conduct a genetic screen in ovarian cancer cells. This library contains 122,417 unique guide RNA sequences targeting 19,052 human genes, and has been shown to be appropriate for the loss-of-function

selection studies (Sanjana, Shalem et al. 2014, Shalem, Sanjana et al. 2014). We used PARP inhibitor sensitivity as a readout and identified candidates that were direct indicators of drug response. The identification of additional HR modifiers and PARPi sensitizers could provide new therapeutic targets to expand the clinical utility of PARP inhibitors.

In this regard, my dissertation focuses on the following two major objectives: (1) to identify experimental therapeutics that enhance the effect of PARP inhibitors (Chapter 2 & 3); and (2) to identify novel therapeutic targets that modify the HR pathway or the response to PARP inhibitor (Chapter 4). For the first objective, I focused on two different strategies that target the FOXM1 pathway (Chapter 2) or the FOXM1-MYC nexus (Chapter 3) to enhance the effects of PARP inhibitor. For the second objective, I focused on TIGAR as a novel therapeutic target to enhance sensitivity to PARP inhibitor.

1.4 Forkhead box protein M1 (FOXM1) and c-MYC in EOC

Forkhead box protein M1 (FOXM1) is a protein encoded by the *FOXM1* gene. It is also known as HNF-3, HFH-11, Trident, and MPP2. The human FOXM1 gene has 10 exons (Lam, Brosens et al. 2013). Alternative splicing of exons Va and VIIa leads to 3 common isoforms of FOXM1: namely, FOXM1a, FOXM1b, and FOXM1c (Figure 1.4.1). FOXM1a does not have transactivation activity due to the presence of both Va and VIIa exons, which disrupt the transactivation domain. FOXM1b does not have either exon Va or VIIa. FOXM1c contains only exon Va. These two isoforms are active transcription factors. FOXM1b has been shown to be the predominantly overexpressed isoform in cancer cells, and functionally more active than FOXM1c (Lam, Ngan et al. 2013). However, studies from our lab (data not shown) and other groups have shown that FOXM1c is the most abundantly expressed isoform in ovarian cancer (Tassi, Todeschini et al. 2017).

FOXM1 is a member of FOX family of transcription factors (Lam, Brosens et al. 2013), which play important roles in regulating the expression of genes involved in cell growth, proliferation and differentiation (Hannenhalli and Kaestner 2009). FOXM1 is highly expressed in S and G_2/M phases (Wierstra and Alves 2007) and has been shown to play crucial roles in G_1-S and G_2-M cell cycle progression and mitotic spindle integrity by regulating expression of many G_2/M-specific genes, including *CCNB2*, *CENPF*, and *PLK2* (Laoukili, Kooistra et al. 2005).

Figure 1.4.1

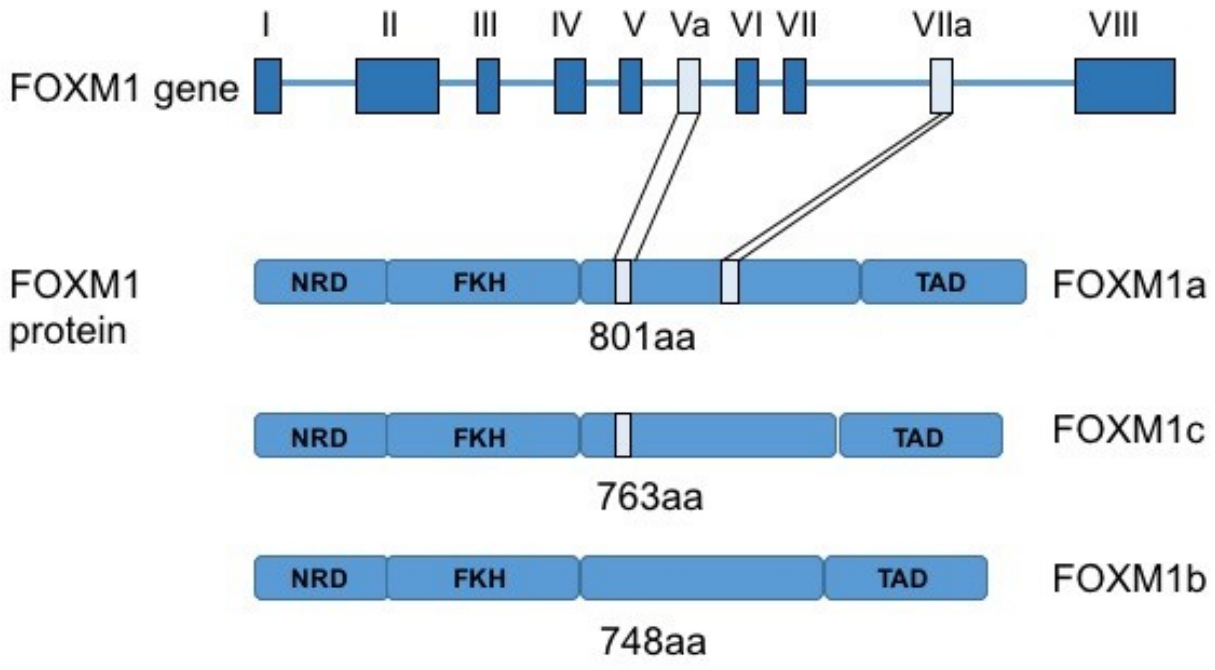


Figure 1.4.1: Alternative splicing of the human FOXM1 gene produces three different isoforms, namely FOXM1a, b, and c. NRD, N-terminal Repressor Domain; FKH, Forkhead winged helix DNA Binding Domain; TAD, Transactivation Domain.

FOXM1 has been implicated in adult tissue homeostasis, embryonic development, and most importantly cancer initiation and progression (Bella, Zona et al. 2014). It is now recognized as a proto-oncogene, and its expression is frequently upregulated in many types of cancer, including liver, prostate, lung, breast, and ovary (Kalinina, Kalinin et al. 2003, Kalin, Wang et al. 2006, Kim, Ackerson et al. 2006, Madureira, Varshochi et al. 2006, Zhang, Cheng et al. 2014). The exact mechanisms of how FOXM1 promotes oncogenesis is still unknown. The fact that FOXM1 overexpression is frequently found at early stages of cancer development suggests FOXM1 plays a key role in cancer initiation (Koo, Muir et al. 2012). It is also shown to facilitate angiogenesis, invasion, and metastasis (Wang, Banerjee et al. 2007), suggesting a potential role in cancer progression. In particular, the FOXM1 expression is essential for countering the oxidative stress induced by the oncogenic HRas mutant (Park, Carr et al. 2009). FOXM1 upregulates the expression of anti-oxidant genes that counteract reactive oxygen species induced by the HRas mutant and prevents cellular senescence. Emerging evidence also indicates that FOXM1 overexpression leads to resistance to the genotoxic therapeutic agent (Wang, Wen et al. 2013, Zhang, Cheng et al. 2014, Tassi, Todeschini et al. 2017), and this effect is attributed to enhanced DNA damage response and DNA repair by FOXM1. FOXM1 transcriptionally regulates the expression of genes involved in DNA damage response and DNA repair, especially HR repair (Tan, Raychaudhuri et al. 2007, Kwok, Peck et al. 2010, Millour, de Olano et al. 2011, Zhang, Wu et al. 2012, Monteiro, Khongkow et al. 2013).

Evaluation of The Cancer Genome Atlas (TCGA) data indicate that FOXM1 transcriptional network is upregulated in over 84% of high-grade serous ovarian cancer tumors, and the FOXM1 pathway positively regulates HR pathway by the direct regulation of *BRCA2* (Cancer Genome Atlas Research 2011). FOXM1 is an attractive target in cancer because of its overexpression in

cancer and its role on the regulation of the HR pathway. However, the effect of FOXM1 inhibition on HR pathway and the role of FOXM1 in PARPi response in cancer cells have not been well characterized.

Thiostrepton is a natural, cyclic oligopeptide antibiotic, which is derived from streptomycetes. Previous studies showed that thiostrepton inhibits FOXM1 transcription factor activity and consequently downregulates FOXM1 expression (Kwok, Myatt et al. 2008, Halasi, Schraufnagel et al. 2009, Hegde, Sanders et al. 2011). Previous studies also showed that thiostrepton enhances the cytotoxicity effect of cisplatin and carboplatin (Zhang, Cheng et al. 2014). However, the combination of thiostrepton and PARP inhibitor was not tested prior to my study, and the findings from my study described in Chapter 2 indicate thiostrepton enhances sensitivity to PARP inhibitor.

c-MYC is a bona fide oncogenic transcription factor encoded by *MYC* proto-oncogene (Dang 2012). It plays important roles in the regulation of cell cycle progression, apoptosis, and most importantly in the cellular transformation to promote genesis of many human cancers (Adams, Harris et al. 1985, Leder, Pattengale et al. 1986, Nilsson and Cleveland 2003, Gabay, Li et al. 2014). As a transcription factor, it activates the expression of many genes by binding to the enhancer box sequence and recruiting histone acetyltransferases (HATs) (Dang, Resar et al. 1999). It can also serve as a repressor through binding with the Miz-1 transcription factor to displace p300 co-activator and inhibit expression of Miz-1 target genes (Schneider, Peukert et al. 1997). It is also reported to have other non-transcriptional function, such as in DNA replication (Dominguez-Sola, Ying et al. 2007) and cap-dependent translation (Cole and Cowling 2008). *MYC* gene amplification occurs in different types of cancer, such as uterine, colorectal, breast, pancreatic, and gastric cancers (Chen, McGee et al. 2014). Importantly, *MYC* gene was found to be amplified in a

significant number of tumor samples from patients with EOC (Ross, Ali et al. 2013). In some cancers, c-MYC expression is highly dependent on BRD4 (Shi and Vakoc 2014), which is a member of bromodomain and extra-terminal motif (BET) family of proteins. Recent studies have shown that c-MYC expression can be successfully blocked by BET inhibitors in cancer cells (Dawson, Prinjha et al. 2011, Delmore, Issa et al. 2011, Mertz, Conery et al. 2011, Zuber, Shi et al. 2011). BET inhibitors are a class of drug that is designed to reversibly bind the bromodomains of BET proteins (BRD2, BRD3, BRD4, and BRDT) to prevent their interactions with acetylated histones and transcription factors (Nicodeme, Jeffrey et al. 2010, Dawson, Prinjha et al. 2011) to decrease gene transcription. BET proteins, especially BRD4, have been shown to drive pathogenesis in various types of cancers, and BET inhibitors have been tested in cancers. In addition, BET inhibitors are also effective in overcoming resistance to other targeted therapies when used in combination therapies (Knoechel, Roderick et al. 2014, Korkut, Wang et al. 2015). Interestingly, BET inhibitors also downregulate FOXM1 expression (Zhang, Ma et al. 2016). However, the relationship between FOXM1 and c-MYC has not been very well characterized, and the potential role of targeting FOXM1-MYC nexus to suppress the expression of DNA repair genes has not been studied. To fill this gap, in Chapter 3, I will describe my studies on the role of FOXM1 and c-MYC in the expression of HR repair genes and to explore the potential of using BET inhibitors to enhance the effect of PARP inhibitors in ovarian cancer.

1.5 Specific Aims of the dissertation

The ultimate goals of these thesis studies were to achieve a better understanding of the underlying mechanisms determining PARP inhibitor responses in ovarian cancer and to identify new strategies that enhance the therapeutic effects of PARP inhibitors. Results from these studies could ultimately advance precision cancer medicine by enabling the translation of genetic knowledge into clinical management of patients with ovarian or breast cancer.

1.5.1 Specific Aim 1: To determine the effects of targeting FOXM1 pathway on PARP inhibitor sensitivity

When aberrantly expressed, FOXM1 can act as an oncogenic transcription factor that has been shown to regulate the expression of genes involved in DNA damage response, DNA repair, and cell cycle progression. The FOXM1 pathway is activated in 84% of high-grade serous ovarian cancer (HGS-OvCa) (Cancer Genome Atlas Research, 2011). Overexpression of FOXM1 is correlated with chemotherapy resistance and poor outcome of patients treated with cytotoxic agents and targeted therapies (Park, Jung et al. 2012, Li, Qiu et al. 2013, Li, Yao et al. 2014, Zhao, Siu et al. 2014). Previous work from our lab has shown that FOXM1 inhibition by thiostrepton induces cytotoxicity and enhances the sensitivity to cisplatin and carboplatin in ovarian cancer (Zhang, Cheng et al. 2014). In this specific aim, I tested my hypothesis that FOXM1 pathway inhibition induces *"BRCAness"* in ovarian cancer cells and sensitizes them to PARP inhibitor. I investigated the extent to which FOXM1 pathway inhibition by siRNAs and FOXM1 inhibitor thiostrepton enhances the sensitivity to olaparib in ovarian cancer cells. Additionally, I explored the molecular mechanisms underlying the enhanced cytotoxic effects of olaparib in FOXM1 pathway-disrupted ovarian cancer cells.

1.5.2 Specific Aim 2: To determine the effects of targeting FOXM1-MYC nexus on PARP inhibitor sensitivity

Both FOXM1 and c-MYC are highly expressed in EOC (Cancer Genome Atlas Research 2011, Ross, Ali et al. 2013). My experimental data show that particular target genes of FOXM1 in HR pathway, *BRCA1, BRCA2, FANCF and BRIP1*, were not downregulated with FOXM1 siRNA. Concomitantly, I observed upregulation of c-MYC protein after the FOXM1 knockdown, suggesting that c-MYC upregulation may serve as a compensation to FOXM1 knockdown. The analysis of ENCODE (Encyclopedia of DNA Elements) data indicates that both FOXM1 and c-MYC bind to overlapping regulatory regions in HR genes, including *BRCA1* and *RAD51*. Considering that BET inhibitors downregulate both c-MYC and FOXM1 pathway, we hypothesize that HR genes are co-regulated by FOXM1 and c-MYC, and inhibition of both with BET inhibitor induces *"BRCAness"* leading to sensitivity to PARP inhibitor in cancer cells.

1.5.3 Specific Aim 3: To identify novel genetic determinants of PARP inhibitor sensitivity by the CRISPR/Cas9 based loss-of-function genetic screen

Based on the concept of synthetic lethality, cancer cells that have lost the ability to repair DSB by HR, including those with defects in BRCA1/2, are highly sensitive to PARP inhibitors (Bryant, Schultz et al. 2005, Farmer, McCabe et al. 2005). These results suggest that functional status of HR pathway is an important determinant of PARP inhibitor responses in cancer cells. However, the extent to which other genetic alterations in cancer can affect how cancer cells will respond to PARP inhibitors is not yet known. The genome-scale CRISPR-Cas9 knockout (GeCKO) library (containing 122,417 unique guide sequences targeting 19,052 human genes) was developed and has been shown to be successful in loss-of-function selection studies (Sanjana, Shalem et al. 2014, Shalem, Sanjana et al. 2014). We propose a working hypothesis that loss-of-function genetic

alterations in HR modifiers modify PARP inhibitor sensitivity and resistance in cancer cells. The objective of these studies is to perform a functional genetic screen to identify novel modifiers of PARP inhibitor response in ovarian cancer cells.

Chapter 2. Targeting FOXM1 pathway disrupts the adaptive response induced by olaparib and enhances sensitivity to PARP inhibitors

2.1 Abstract

FOXM1 transcription factor network is activated in over 84% of cases in high-grade serous ovarian cancer, and FOXM1 upregulates the expression of genes involved in homologous recombination (HR) repair pathway. However, the role of FOXM1 in poly (ADP-ribose) polymerase (PARP) inhibitor response has not yet been studied. In the present study, we showed that PARP inhibitor olaparib induced the expression and nuclear localization of FOXM1. Using ChIP-qPCR, we demonstrated that olaparib treatment increased the binding of FOXM1 to genes involved in HR repair. FOXM1 knockdown by RNAi or inhibition by thiostrepton decreased FOXM1 expression and the expression of HR repair genes, such as *BRCA1* and *RAD51*. Consequently, both FOXM1 knockdown and inhibition by thiostrepton enhanced sensitivity to olaparib. Comet assay and PARP trapping assay showed increased DNA damage and PARP trapping in FOXM1 inhibited cells treated with olaparib. Finally, thiostrepton decreased the expression of BRCA1 in rucaparib-resistant cells and enhanced sensitivity to rucaparib. Collectively, these results suggest that FOXM1 plays an important role in the adaptive response induced by olaparib and FOXM1 inhibition by thiostrepton induces *"BRCAness"* and enhances sensitivity to PARP inhibitors. In conclusion, FOXM1 inhibition represents an effective strategy to overcome resistance to PARP inhibitors, and thus targeting FOXM1-mediated adaptive pathway may produce better therapeutic effects for this class of inhibitors.

2.2 Introduction

The primary cause of cancer-related mortality is the treatment failure resulting from intrinsic or acquired resistance to chemotherapy (Giaccone and Pinedo 1996, Yardley 2013, Hammond, Swaika et al. 2016). In epithelial ovarian cancer, although most patients initially respond to chemotherapy, they experience recurrences and the acquired resistance to chemotherapy (Pfisterer and Ledermann 2006). Consequently, epithelial ovarian cancer is the most lethal gynecologic malignancies in the United States (Siegel, Miller et al. 2015). Although overall survival from ovarian cancer has improved slowly over the past three decades (Bast, Hennessy et al. 2009), recent advances in PARP inhibitors as maintenance therapies are having positive impacts on the overall survival of patients with ovarian cancer (Bowtell, Bohm et al. 2015). Nonetheless, acquired resistance to PARP inhibitors are being reported (Fojo and Bates 2013, Johnson, Johnson et al. 2013), and it is important to understand molecular mechanisms that contribute to acquired resistance to chemotherapeutic agents.

Intrinsic and acquired resistance to chemotherapeutic agents can be explained by the Darwinian selection of fitness-conferring genetic traits under drug treatment (Gerlinger and Swanton 2010). Under this principle, cells with pre-existing mutations that confer fitness under drug treatment are selected, thereby contributing to the development of resistance to treatment. Consistent with this principle, low-level revertant mutations in *BRCA1* and *BRCA2* are found in ovarian carcinoma samples prior to platinum-based chemotherapy, and these rare mutations become enriched in carcinoma samples from the corresponding patients during relapse (Patch, Christie et al. 2015), suggesting the selection of pre-existing fitness-conferring somatic mutations by chemotherapy. Although this principle explains intrinsic resistance, it cannot fully explain the acquired resistance.

With respect to the acquired resistance in ovarian cancer, a subset of patients generally respond to platinum-based chemotherapy even after relapse from prior platinum-based chemotherapy (Pfisterer and Ledermann 2006). Recent evidence suggests adaptive cellular response may provide a transitional state that allows cells to acquire fitness-conferring genetic mutations after several rounds of treatment with chemotherapeutic agents (Goldman, Majumder et al. 2015). A non-genetic Lamarckian mechanism of drug-induced adaptive response has been proposed as a possible mechanism for the acquisition of resistance (Pisco, Brock et al. 2013). The 'transient adaptive resistance' allows cells to be in 'chemotherapy-tolerant state', thereby produces 'persisters' (Dawson, Intapa et al. 2011, Goldman, Majumder et al. 2015). Extracellular matrix and tumor microenvironment have been shown to provide such transient adaptive resistance to cancer cells (Chien, Kuang et al. 2013). These 'persisters' subsequently acquire fitness-conferring genetic and epigenetic alterations that promote resistance to chemotherapy (Sharma, Lee et al. 2010). Consistent with this concept, recent studies indicate that *in vitro* selection with the PARP inhibitor rucaparib in MDA-MB-436 breast cancer cells resulted in resistant clones that overexpressed mutant BRCA1 at higher levels than in drug-sensitive parental MDA-MB-436 (Johnson, Johnson et al. 2013). In this model, epigenetic rather than genetic alterations contribute to the acquired resistance to rucaparib. In the population of cells without preexisting genetic alterations that confer fitness under drug treatment, the adaptive cellular response may represent a critical step prior to the acquisition of acquired resistance. Therefore, adaptive cellular responses may be targeted to overcome acquired resistance to chemotherapeutic agents.

A critical step in the development of effective combination therapies to extend the efficacy of existing chemotherapeutic agents is to understand the molecular mechanisms regulating the adaptive cellular responses to existing chemotherapeutic agents. According to the landmark study

by TCGA scientists, the FOXM1 pathway is activated in approximately 84% of high-grade serous ovarian carcinomas (Cancer Genome Atlas Research 2011). FOXM1 regulates the expression of DNA repair genes (Tassi, Todeschini et al. 2017) as well as genes involved in adaptive response to cellular stress induced by oxidative stress and oncogenic stress (Park, Carr et al. 2009). However, the extent to which FOXM1 pathway contributes to the adaptive cellular response to chemotherapy and represents an important epigenetic molecular mechanism regulating the adaptive cellular response to chemotherapy is not yet characterized.

In this study, the FOXM1 pathway was identified as a component of the adaptive cellular response to PARP inhibitor olaparib. We found that olaparib induced FOXM1 expression which regulates several genes involved in homology recombination repair pathway. RNAi or FOXM1 inhibitor thiostrepton decreased FOXM1 expression and attenuated the adaptive cellular response leading to enhanced sensitivity to olaparib. Finally, our results showed that FOXM1 inhibitor thiostrepton decreases the expression of *BRCA1* and *BRCA2*, produces *"BRCAness"*, and enhances sensitivity to olaparib. Our results support an emerging paradigm that adaptive cellular responses may be targeted to prevent acquired resistance to chemotherapeutic agents and indicate that FOXM1 pathway may be targeted to prevent acquired resistance to PARP inhibitors.

2.3 Materials and Methods

Cell lines and cell culture

ES-2, OVCAR3 and A2780 cells were maintained in MCDB105 and M199 (1:1) (Sigma, USA) containing 5% FBS (Sigma), OV90 cells were maintained in MCDB105 and M199 (1:1) with 15% FBS. OVCA420* cells were cultured in DMEM (Sigma and Caisson Labs, USA) supplemented with 10% FBS. ONCO-DG1 cells were grown in RPMI1640 (Sigma and Caisson Labs) with 10%

FBS. MDA-MB-436 and its derivative rucaparib resistant cells RR-1, RR-2, RR-3 were kind gifts from Dr. Neil Johnson laboratory at Fox Chase Cancer Center (Johnson, Johnson et al. 2013) and were maintained in RPMI1640 (Sigma and Caisson Labs) supplemented with 10% FBS. All the media were supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin. ES-2, OVCAR3, OVCA420* and OV90 cells were gifts from Dr. Viji Shridhar (Mayo Clinic). A2780 cells were provided by Dr. Andrew Godwin (The University of Kansas Medical Center). ONCO-DG1 was purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. All cell lines were subjected to cell line identity confirmation. All experiments performed on cells that were passaged less than 20 times. Mycoplasma testing was performed during the studies, and cell cultures were free of mycoplasma. Cell line identification was performed at the end of experiments. OV90 and ONCO-DG1 showed 100% STR profiles matching to corresponding cell lines reported in ATCC or ExPASy. STR profiles for ES-2 and OVCAR3 were performed in 2014 as a supplement to our recent publication (Zhang, Cheng et al. 2014). STR profiles for OVCA420* does not match with any reported cell lines in ATCC, ExPASy, DSMZ, or CLIMA, and therefore we placed an asterisk to differentiate it from the original cell line.

Antibodies and Compounds

Rabbit polyclonal anti-FOXM1 antibody (C-20, sc-502), rabbit polyclonal anti-BRCA1 antibody (C-20, sc-642), mouse monoclonal anti-FANCF antibody (D-2, sc-271952) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit polyclonal anti-Histone H3 antibody (ab1791) and rabbit monoclonal anti-β-Tubulin antibody (ab108342) were obtained from Abcam (Cambridge, MA, USA). Mouse monoclonal anti-RAD51 antibody (5B3/6, GTX23638), rabbit polyclonal anti-Histone H3 antibody (GTX122148) and rabbit polyclonal anti-BRCC3 antibody (GTX31765) were from GeneTex (Irvine, CA, USA). Rabbit polyclonal anti-PARP antibody

(9542S) and rabbit monoclonal anti-Caspase 3 antibodies (9665S) were purchased from Cell Signaling Technologies (Danvers, MA, USA). Mouse monoclonal anti-Poly (ADP-ribose) antibody (PAR, 10H, ALX-804-220-R100) was obtained from Enzo Life Sciences (Alexis, San Diego, USA). Mouse monoclonal anti-beta actin antibody (A1978) was from Sigma-Aldrich (St Louis, MO, USA). For Secondary antibodies, horse anti-mouse IgG-HRP antibody (7076S) was purchased from Cell Signaling Technologies, Goat anti-rabbit IgG-HRP antibody (sc-2030) was from Santa Cruz Biotechnology. Olaparib (AZD2281, Ku-0059436) was purchased from Selleckchem (TX, USA). Olaparib stock solutions were made with DMSO at 50 mM and stored at -80°C. Thiostrepton powder was purchased from Santa Cruz Biotechnology (sc-203412A) and formulated as micelle-encapsulated thiostrepton (see supplement information, Materials and Methods 2) by Dr. Laird Forrest (School of Pharmacy, University of Kansas, Lawrence, KS, USA).

Immunoblotting

Cells were washed at least twice with PBS at the end of treatments if applicable and then lysed with an appropriate volume of 1X electrophoresis sample buffer (Bio-Rad Laboratories, CA, US) with 5% β-mercaptoethanol (Sigma-Aldrich). The cell lysates were then boiled at 95 °C for 5 minutes before using. Immunoblotting procedures were performed as previously described (Zhang, Cheng et al. 2014). For apoptosis marker check, cells were collected at the end of treatments, and total proteins were extracted using RIPA buffer (1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS in 1 X PBS) containing protease/phosphatase inhibitor cocktail (Roche). BCA protein assay reagent kit (Pierce) was used to determine protein concentrations. Equal amounts of total proteins were loaded for SDS-PAGE and transferred onto PVDF membranes (GE healthcare). For nuclear fractionation assays, cell pellets were collected and lysed with cytoplasmic extraction buffer (CEB) in Subcellular Protein Fractionation Kit (78840, Thermo

Scientific, Rockford, IL, USA) and then centrifuged at 500 X g for 5 minutes, the supernatant was labeled as the cytoplasmic extract, while the pellets were further lysed in RIPA buffer. Protein concentration was determined by the BCA assay and equal amounts of proteins were loaded for western blot analysis. The densitometric analysis was performed with Image J software (NIH).

PARP trapping assay

4.5 x 10^5 cells were treated with the appropriate drug(s) for 4 hours before collection. The cell pellets were fractionated using Subcellular Protein Fractionation kit (78840, Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions and subjected to immunoblotting.

Chromatin Immunoprecipitation (ChIP)-qPCR

Chromatin immunoprecipitation was carried out as described before (Johnson, Mortazavi et al. 2007). Briefly, after 20 μM Olaparib treatment for 12 hours or 24 hours, cells were cross-linked with 1% formaldehyde (Electron Microscopy Sciences, USA) for 10 min and quenched by cross-linking by glycine. The chromatin was sonicated with a Bioruptor Twin (Diagenode) at maximum setting for 12 min. The sonicated chromatin with incubated with 1.0 μg FOXM1 antibody (C-20, sc-502, Santa Cruz Technology, USA) at 4°C for 2-4 hours before purification with 100 μL Protein A/G magnetic beads (88803, Pierce). The beads were washed 5 times with LiCl wash buffer (100 mM Tris pH7.5, 500 mM LiCl, 1% NP-40, 1% sodium deoxycholate before washed with 1X TE buffer (10 mM Tris-HCl pH7.5 and 0.1 mM Na_2EDTA) and eluted with Elution Buffer (1% SDS and 0.1 M $NaHCO_3$). After reverse-crosslinking, the DNA was purified with the QIAQuick PCR cleanup kit (QIAGEN) and used for qPCR, which was performed on a CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad) using RT^2 SYBR Green qPCR Mastermix (QIAGEN). The sequences of the primer pairs were listed in Table 2.3.1.

Real-time quantitative PCR (RT-qPCR)

The total RNA was extracted with the Trizol reagent (15596-028, Invitrogen) according to the manufacturer's manual. The cDNA was synthesized using SuperScript II reverse transcriptase (180604014, Invitrogen) with 1 μg of total RNA in a 20 μL reaction. The resulting cDNA was diluted 1:20 in nuclease-free water and 1 μL was used per qPCR reaction with triplicates. QPCR was carried out using Power SYBR Green PCR Master Mix (4367659, Thermo Fisher Scientific) on a CFX384 Real-Time PCR Detection System (Bio-Rad) including a non-template negative control. Amplification of GAPDH or 18S rRNA was used to normalize the level of mRNA expression. The sequences of the primer pairs were listed in Table 2.3.1.

Table 2.3.1

Table 2.3.1

Gene	Forward	Reverse
FOXM1(total)	5′AGAATTGTCACCTGGAGCAG	5′TTCCTCTCAGTGCTGTTGATG
FOXM1A	5′GGTACACCCATCACCAGCTT	5′ATGGGTCTCGCTAAGTGTGG
FOXM1B	5′CGTGGATTGAGGACCACTTT	5′TCGGTCGTTTCTGCTGCTT
FOXM1C	5′CCCGAGCACTTGGAATCAC	5′TCCTCAGCTAGCAGCACCTT
CCNB1	5’GGCTTTCTCTGATGTAATTCTTGC	5’GTATTTTGGTCTGACTGCTTGC
FANCF	5’GCATTTGGGTTGGAACTGAG	5’CTTCAAAATCTCCATCCTGCG
BRCA1	5’TAATGCTATGCAGAAAATCTTAGAG	5’TACTTTCTTGTAGGCTCCTTTTGG
BRCA2	5’TTCATGGAGCAGAACTGGTG	5’AGGAAAAGGTCTAGGGTCAGG
BRCC3	5’CCTCATGTCACTATCGGGAAAG	5’GGATCTTGGTTACTGAGTCCAG
BRIP1	5’GCTTAGCCTTACTTTGTTCTGC	5’TTTCACTTACGCCCTCATCTG
NBS1	5’AGACCAACTCCATCAGAAACTAC	5’AATGAGGGTGTAGCAGGTTG
CsK1	5GAATGGAGGAATCTTGGCGTT	5’TCTTTGGTTTCTTGGGTAGTGGG
Skp2	5’CTGGGTGTTCTGGATTCTCTG	5’GCTGGGTGATGGTCTCTG
DDIT3	5’GTACCTATGTTTCACCTCCTGG	5’TGGAATCTGGAGAGTGAGGG
DDIT4	5’GTTTGACCGCTCCACGAG	5’GTGTTCATCCTCAGGGTCAC
GADD45A	5’GGAGAGCAGAAGACCGAAAG	5’AGGCACAACACCACGTTATC
BCL-2	5’GTGGATGACTGAGTACCTGAAC	5’GCCAGGAGAAATCAAACAGAGG
GAPDH	5’GAAACTGTGGCGTGATGGC	5’CACCACTGACACGTTGGCAG
18S rRNA	5’GCCCGAAGCGTTTACTTTGA	5’TCCATTATTCCTAGCTGCGGTATC
CCNB1 FOXM1 BS	5’ CGCGATCGCCCTGGAAACGCA	5’CCCAGCAGAAACCAACAGCCGT
BRCA1 FOXM1 BS	5’CAAGGTACAATCAGAGGATGGG	5’TCCTCTTCCGTCTCTTTCCT
RAD51 FOXM1 BS	5’ACCAGGCAGAGAATCTTGTTC	5’TTCAAGTCTAACCCAGTGCAG
FANCF FOXM1 BS	5’AAGGCCCTACTTCCGCTTTC	5’CACGGATAAAGACGCTGGGA
RAD51D FOXM1 BS	5’CACATTCGGCCTCTACCTTC	5’TTGGAACGGAAGCTGGC
FANCD2 FOXM1 BS	5’TTGGCGTCACGTCATGG	5’CCACTTACTCACCGAGAAGC

siRNA transfection

FOXM1 specific siRNAs and scrambled negative control siRNAs were synthesized by Integrated DNA Technologies (IDT, Coralville, IA, USA). 3.5 x 10^5 cells/well were seeded in 6-well plates and incubated at 37 °C overnight. Next day, 20 nM of each siRNA was transfected into the cells with Oligofectamine Transfection Reagent (12252011, Invitrogen) according to manufacturer's instructions. Culture media was added 6-8 hours after transfection without washing cells. 48 hours after transfection, the transfected cells were trypsinized and seeded on 96-well plates (for cytotoxicity assay) or 6-well plates (for colony formation assay). Drugs were added around 12 hours after seeding. For cytotoxicity assay, cells were incubated with drug for 3 days before measurement of cell viability using sulforhodamine B assay. For colony formation assay, cells were exposed to drugs for 3 days and then changed to fresh media without drug until colonies formed and stained with sulforhodamine B for imaging. To check the downregulation of FOXM1 expression, the transfected cells were collected to extract total RNA for qRT-PCR or proteins for western blot analysis 72 hrs after transfection. The sequences of siRNAs used are listed below:

FOXM1 siRNA#1: sense rGrUrGrCrCrArArCrCrGrCrUrArCrUrUrGrArCrArUrUrGGA,
antisense rUrCrCrArArUrGrUrCrArArGrUrArGrCrGrGrUrUrGrGrCrArCrUrG;
FOXM1 siRNA#2: sense rGrCrGrCrUrArUrUrArGrArUrGrUrUrUrCrUrCrUrGrArUAA,
antisense rUrUrArUrCrArGrArGrArArArCrArUrCrUrArArUrArGrCrGrCrArC.

Cytotoxicity assay using Sulforhodamine B (SRB) and drug synergy studies

SRB assays were performed as previously described (Vichai and Kirtikara 2006, Bastola, Neums et al. 2016) with modifications shown below. For OVCA420*, OV90, ES-2, ONCO-DG1,

OVCAR3 cells, 3000 cells/well were seeded in 96-well plates and treated with drugs at least 12 hours after seeding. Then the cells were incubated for another 3 days. For MDA-MB-436 rucaparib resistant cells RR-1, RR-2, and RR-3, 5000 cells were seeded and incubated for 5 days after drug treatment. Dose-response curves were fitted and the IC_{50} for each drug was determined using GraphPad Prism 6 four parameters. All curves were constrained with 100% on top. Synergy was determined by calculating the combination index (CI) obtained from the plate reading. CI was calculated based on dividing the expected effect by the observed effect.

Colony formation assay

For OVCA420*, OV90, and ES-2, 1000 cells were seeded in 6-well plates. For MDA-MB-436 rucaparib resistant cells RR-1 and RR-2, 2000 or 3000 cells were seeded per well in 6-well plates. The cells were treated with drugs at least 12hours after seeding and further incubated for another 3 days before changing to fresh media. The medium was changed every 2-3 days to allow colonies to form. At the end of experiments, SRB assay was performed to stain the colonies which were imaged with Molecular Imager ChemiDoc MP System (Bio-Rad). The colonies were further dissolved and measured with a plate reader. Analysis of colonies was performed in GraphPad Prism 6.

Caspase 3 activity assay

Caspase 3 activity assay was performed as previously described (Bastola, Neums et al. 2016). Briefly, 4 x 10^5 cells/well were seeded in 6-well plates and incubated overnight. The next day, cells were treated with appropriated drugs and incubated for 30 hours. Cells were then collected using a cell lifter and lysed in caspase buffer (pH 7.2, 20 mM PIPES, 100 mM NaCl, 1 mM EDTA (pH 8.0), 0.1% (w/v) CHAPS, 10% sucrose and 10 mM DTT) and quantified with BCA assay. 20

μg of total protein were used to combine with 2 μL of 2 mM DEVD-Afc (Millipore) in 96-well flat-bottom plates and added 200 μL/well caspase buffers. The plate was covered and incubated war 37 °C for 2 hours before measuring fluorescence at Excitation of 400 nm and Emission of 510 nm. Measurements were analyzed using GraphPad Prism 6.

Alkaline Comet assay

We used the CometChip Electrophoresis Starter kit (TREVIGEN, 4260-096-ESK) to perform alkaline comet assay according to manufacturer's instructions. For thiostrepton and olaparib combination experiment, cells were treated with vehicle or thiostrepton for 4 hours before seeding onto equilibrated 96-well CometChip. Then 100 μL/well of fresh culture media containing appropriate concentrations of both Thiostrepton and Olaparib was added to CometChip followed by another 4 hours' incubation at 37°C. And alkaline comet assay was performed following the product instruction. For *FOXM1* siRNA transient knockdown experiment, cells were transfected with scrambled siRNA or *FOXM1* siRNA and waited 72 hours before seeding onto CometChip. Cells were then incubated with vehicle or Olaparib for 4 hours at 37 °C before alkaline comet assay. Comets were analyzed with Trevigen Comet Analysis Software after imaging under a 4X fluorescent microscope.

Statistical analysis

All data were analyzed using GraphPad Prism 6. Results were expressed as means ± standard error of the mean (SEM). Differences between treatment regimens were analyzed by one-way ANOVA or two-tailed Student's t-test. $p \leq 0.05$ was considered to be statistically significant.

2.4 Results

2.4.1 Olaparib induces the expression of FOXM1 and HR repair genes

To establish the potential role of FOXM1 in the adaptive cellular response induced by olaparib, Ovarian cancer cells ES-2 and OVCA420* were treated with olaparib and determined the expression of FOXM1, BRCA1, and RAD51 at different time points. We observed induction of FOXM1 expression by olaparib within six hours of treatment (Figure 2.4.1.A). In addition to the upregulation of FOXM1 expression, the increased nuclear location of FOXM1 was observed within three hours of olaparib treatment (Figure 2.4.1.B), indicative of FOXM1 pathway activation. Concomitant with FOXM1 pathway activation, we observed increased expression of BRCA1 and RAD51 in these cells (Figure 2.4.1.C-D). Consistent with the increase in FOXM1 nuclear localization, FOXM1 binding to promoter regions of its target genes, such as *BRCA1*, *RAD51*, *FANCF*, *RAD51D*, and *FANCD2*, increased at 12 hours and 24 hours after olaparib treatment (Figure 2.4.1.E). These data suggest that FOXM1 plays an important role in adaptive cellular response to olaparib treatment in ovarian cancer cells.

Figure 2.4.1

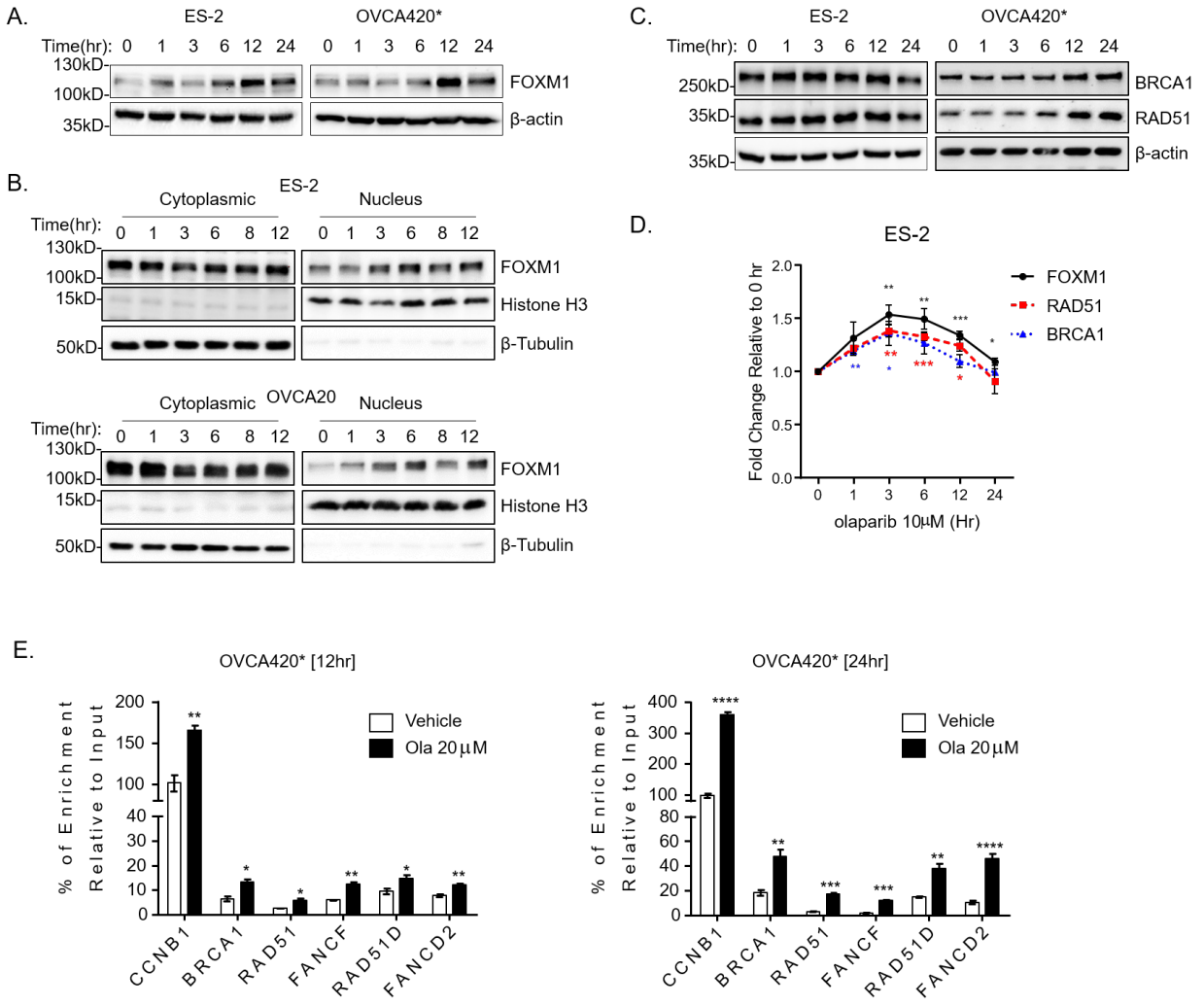


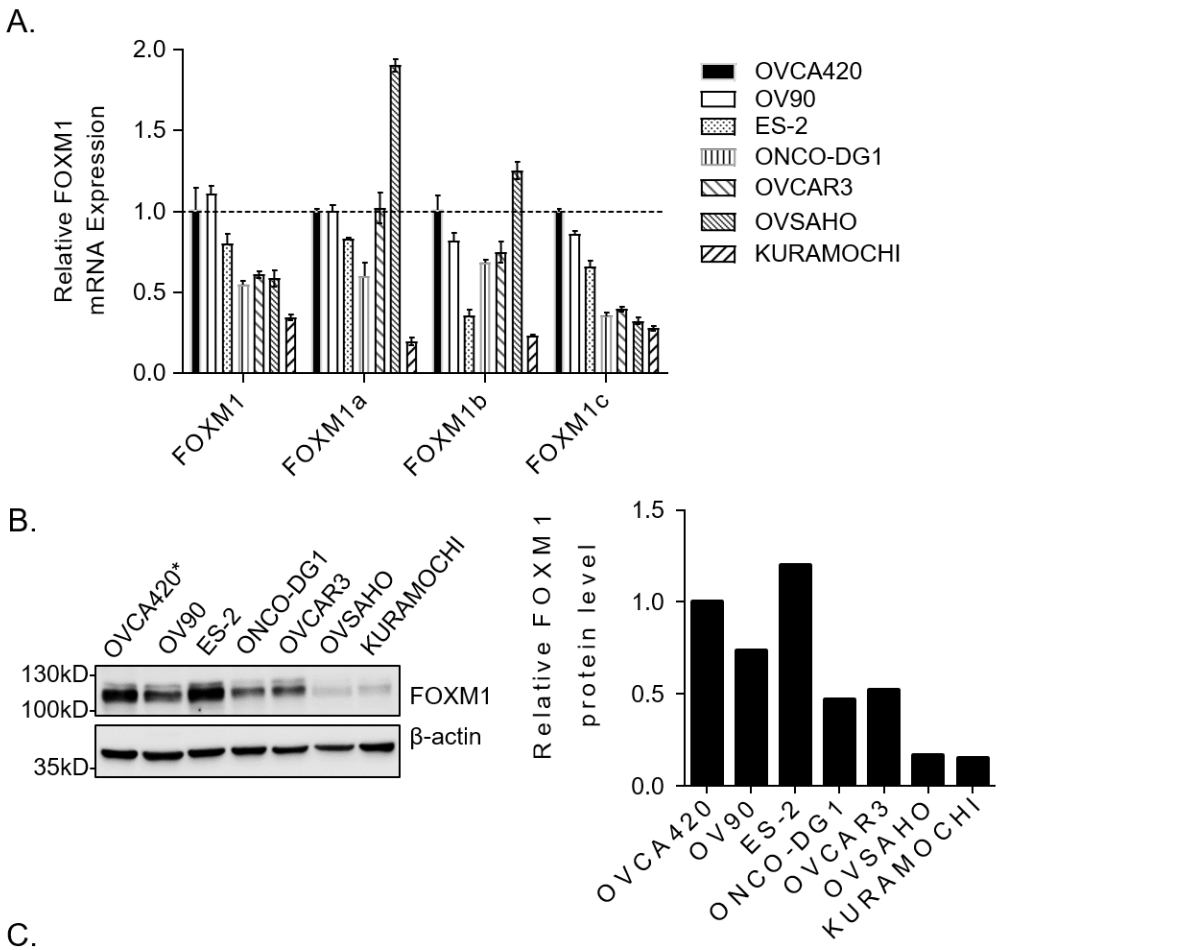
Figure 2.4.1: Olaparib treatment increases FOXM1 expression. A) Olaparib induces FOXM1 expression. The ES-2 and OVCA420* cells were treated with 10 μM olaparib for 0, 1, 3, 6, 12 or 24 hours and subjected to immunoblotting with the FOXM1 antibody. β-actin immunoblot is used as a loading control. **B)** Olaparib enhances nuclear localization of FOXM1. Cells were treated with 10 μM olaparib for 0, 1, 3, 6, 8 or 12 hours before subcellular fractionation followed by Western blot of FOXM1 in nuclear and cytoplasmic fractions. Beta-tubulin and Histone H3 were used as loading controls for cytoplasmic and nuclear protein, respectively. **C)** Olaparib treatment is associated with increases in the expression of BRCA1 and RAD51. The whole cell lysates were prepared from both cell lines after exposure of 10 μM

olaparib for 0, 1, 3, 6, 12 or 24 hours and blotted with BRCA1 or RAD51 antibodies. **D)** RAD51 and BRCA1 protein levels changes with FOXM1 level changes. Quantification of protein levels of FOXM1, RAD51 and BRCA1 in ES-2 cells treated with olaparib for 0, 1, 3, 6, 12 and 24 hours. Data are shown as mean ± SEM from 3 independent experiments. Statistics was done with Student's test. *$p<0.05$, **$p<0.01$, ***$p<0.001$. **E)** Olaparib treatment is associated with increases of FOXM1 occupancy at promoter regions of its target genes. OVCA420* cells were treated with 20 μM olaparib for 12 hours or 24 hours before ChIP analysis using FOXM1 antibody. Results are representative of at least three experiments.

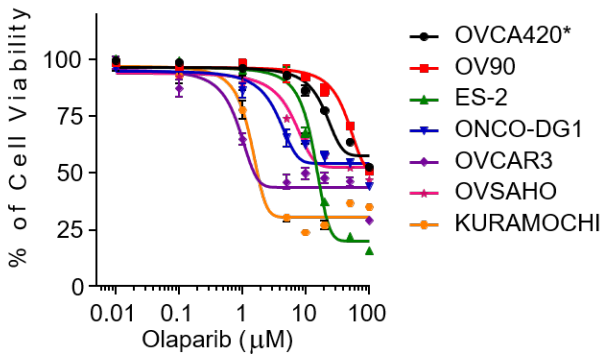
2.4.2 FOXM1 expression is higher in cancer cells that are less responsive to olaparib

To determine the extent to which FOXM1 expression may be correlated with olaparib sensitivity, we assessed *FOXM1* expression by qRT-PCR, the Western blot analysis, and olaparib sensitivity by SRB assay. Although all three forms of *FOXM1* transcripts were expressed (Figure 2.4.2A), protein expression was reflected better by the levels of a *FOXM1C* transcript (Figure 2.4.2B). Consistent with these results, analysis of The Cancer Genome Atlas ovarian cancer dataset indicated that *FOXM1C* transcript is the most abundant among the three isoforms (data not shown), which is consistent with a report from Tassi et al (Tassi, Todeschini et al. 2017). The dose-response curves for olaparib sensitivity in these cell lines indicated that OVCA420* and OV90 with high expression of *FOXM1* were less responsive to olaparib (Figure 2.4.2 C). Taken together, these results suggest a potential role of FOXM1 in olaparib sensitivity in ovarian cancer cells.

Figure 2.4.2



C.



Estimated IC50 for Olaparib (μM)	
OVCA420*	>100μM
OV90	~100μM
ES-2	13.12μM
ONCO-DG1	Not predictable
OVCAR3	0.8μM
OVSAHO	Not predictable
KURAMOCHI	1.3μM

Figure 2.4.2: FOXM1 expression is higher in cancer cells that are less responsive to olaparib. A) qRT-PCR to quantify mRNA level of *FOXM1* isoform a, b, and c in different ovarian cancer cells. mRNA expression was normalized to OVCA420* cell. Data are shown as mean ± SEM. **B)** Left: western blot analysis of FOXM1 expression in different cells. Right: quantification of FOXM1 protein levels. The FOXM1 level was normalized to beta-actin for each cell, which was normalized to normalized FOXM1 level in OVCA420* cell. **C)** Cell viability assay to test olaparib sensitivity in different cells. 3-day SRB assay was done in 96 well plates. Data were plotted in line graph in Prism 6. Data are shown as mean ± SEM. N=3-4. Estimated IC_{50} values were obtained in Prism 6 and shown on the right. "Not predicted" represents that the IC_{50} cannot be accurately predicted.

2.4.3 FOXM1 knockdown results in enhanced sensitivity to olaparib

To determine the extent to which FOXM1 expression contributes to olaparib sensitivity, we used two different siRNAs to downregulate *FOXM1* expression. Although both siRNAs downregulated all three isoforms of *FOXM1* transcripts, *FOXM1B* was affected more by these siRNAs (Figure 2.4.3.A). Interestingly, FOXM1 expression was markedly decreased at the protein level, indicating that these siRNAs also affected the translation of FOXM1 protein (Figure 2.4.3.B); consistent with the translational repression of certain siRNAs (Aleman, Doench et al. 2007). SRB assays indicated that cells with decreased FOXM1 expression were significantly more sensitive to olaparib (Figure 2.4.3.C). Similarly, we observed a significant decrease in clonogenic survival in FOXM1 knocked-down cells treated with 4, 10 and 25 μM olaparib compared to scrambled control siRNA (scr siRNA) (Figure 2.4.3.D).

Figure 2.4.3

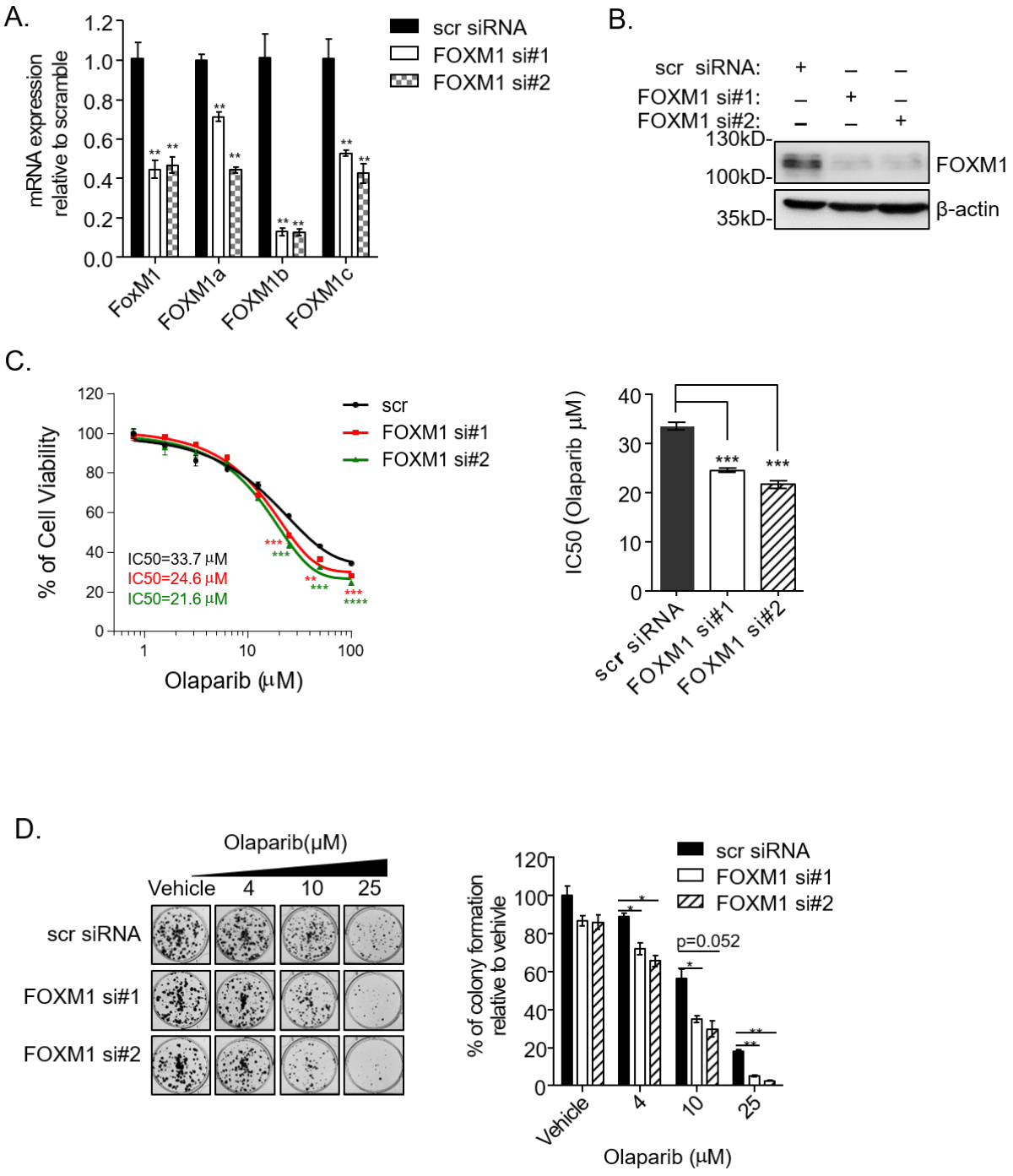


Figure 2.4.3: *FOXM1* knockdown results in enhanced sensitivity to olaparib. A) qRT-PCR analysis to measure the knockdown efficiency of two independent *FOXM1* siRNAs in ES-2 cells. All three *FOXM1*

isoforms were downregulated with both siRNAs at 72 hours. Data are shown as mean ± SEM. *FOXM1* shown as expression relative to scr siRNA transfected cells. Knockdown was performed with two independent siRNAs. **B)** Western blot analysis to check FOXM1 protein with siRNA transfection in ES-2 cells. Beta-actin was used as loading control. **C)** *FOXM1* siRNA knockdown leads to increase sensitivity to olaparib in ES-2 cell by SRB assay. Left: SRB assay to test cell viability after siRNA transfection. 48 hours after transfection, cells were treated with increasing concentrations of olaparib for additional 3 days. Right: Bar graph shows decreased IC_{50} of olaparib in *FOXM1* siRNA knockdown cells. Results were derived from triplicates of representative experiments. **D)** Clonogenic assay with siRNA knockdown in ES-2 cell. Left: Representative images of colonies in 6 well plate. Cells were treated with increasing concentrations of olaparib for 3 days and kept for 2 weeks without drug to allow colonies to form. Data are shown as mean ± SEM. Statistics was done using the Student's t-test. $p<0.05$ was considered to be significant. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

2.4.4 FOXM1 inhibition with thiostrepton increases sensitivity to olaparib

Next, we used pharmacologic means to inhibit FOXM1 expression and determined the extent to which FOXM1 inhibition results in enhanced sensitivity to olaparib. Thiostrepton, is a large cyclic antibiotic commonly used in topical antibiotic ointments. It is known to inhibit bacterial protein synthesis and block translation by interfering with elongation factors (EF) involved in ribosomal GTPase activity. In addition to these activities, previous studies have shown that thiostrepton can also repress the *FOXM1* promoter (Kwok, Myatt et al. 2008) and in turn downregulate FOXM1 expression (Hegde, Sanders et al. 2011, Zhang, Cheng et al. 2014). In three different ovarian cancer cell lines, we observed synergistic interactions between thiostrepton and olaparib at the concentrations tested in clonogenic assays (Figure 2.4.4.A-B) and mild synergistic activity in SRB assays (Figure 2.4.4.C). Collectively, these results indicated that FOXM1 inhibition enhances sensitivity to olaparib.

Figure 2.4.4

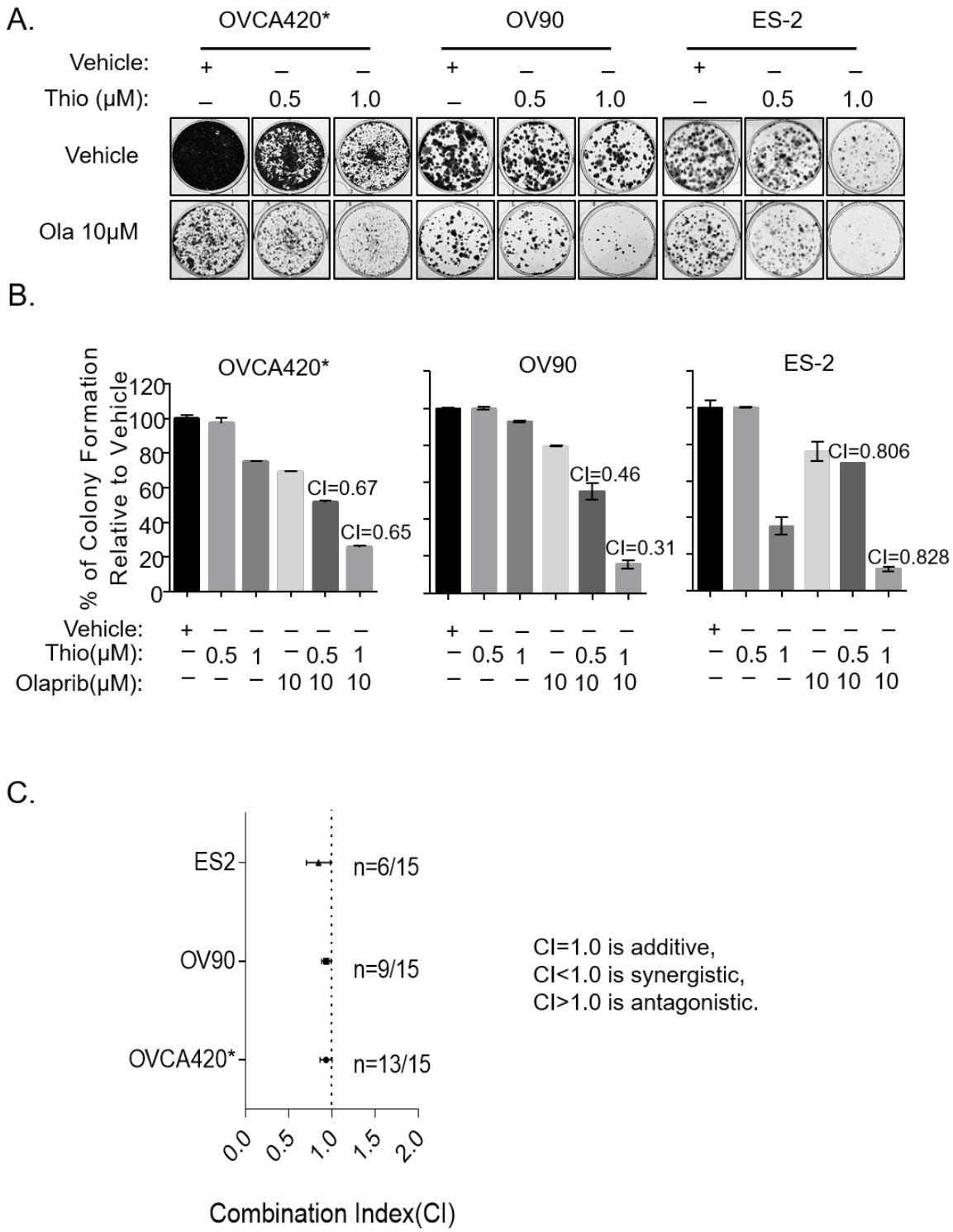

A.
OVCA420*
OV90
ES-2
Vehicle:
Thio (μM):
Vehicle
Ola 10μM
B.
% of Colony Formation Relative to Vehicle
OVCA420*
OV90
ES-2
CI=0.67
CI=0.65
CI=0.46
CI=0.31
CI=0.806
CI=0.828
Vehicle:
Thio(μM):
Olaprib(μM):
C.
ES2
OV90
OVCA420*
n=6/15
n=9/15
n=13/15
Combination Index(CI)
CI=1.0 is additive,
CI<1.0 is synergistic,
CI>1.0 is antagonistic.

Figure 2.4.4: FOXM1 inhibition with thiostrepton increases sensitivity to olaparib. A) Colony formation assay to test the combinatory effect of thiostrepton and olaparib treatment. Representative images of colonies were shown. Cells were seeded in 6 well plates and exposed to vehicle, thiostrepton, olaparib or thiostrepton & olaparib for 3 days, and changed to fresh media without drugs every 3 days and kept for 2 weeks to allow colonies to form. **B)** Quantification of colonies indicates a synergistic interaction between thiostrepton and olaparib. Data presented as colony formation relative to the vehicle group. Data are shown as mean ± SEM. Combination index (CI) was calculated with a function as: $((E_{thio}+(1-E_{thio})*E_{ola})/E_{observed}$, where E_{thio}, E_{ola}, and $E_{observed}$ represent drug effect of thiostrepton alone, olaparib alone, and the observed effects of the combination of both drugs, respectively. The two drugs are synergistic if CI<1.0, additive if CI=1.0, and antagonistic if CI>1.0. CI values were indicated in the graph. **C)** Mild synergistic interactions between thiostrepton and olaparib by SRB assay. CIs for different combinations were calculated and CIs resulted from drug effects between 0.2-0.8 were used in the plot. Data are shown as mean with 95% confidence intervals.

2.4.5 Thiostrepton decreases the expression of DNA repair genes and increases the expression of pro-apoptotic genes

To better characterize the effect of thiostrepton and define the molecular pathways affected by thiostrepton, our lab analyzed the publicly available dataset from the Connectivity Map (https://portals.broadinstitute.org/cmap/fileDownloadServlet?servletAction=input) project where cancer cells were treated with thiostrepton for six hours and drug-perturbed transcriptomes were profiled with array-based gene expression analysis.(Lamb, Crawford et al. 2006). The Metascape analysis (Tripathi, Pohl et al. 2015) of genes that are downregulated by thiostrepton at p<=0.001 indicates these genes are associated with mitotic cell cycle, G1/S phase transition and FOXM1 pathway (Fang, Madden et al. 2018). On the other hand, the Metascape analysis of genes that are upregulated by thiostrepton at p<=0.001 indicates these genes are associated with unfolded protein response, ER-associated degradation, and cellular redox homeostasis. At p <= 0.005, we observed 716 genes that are differentially expressed between DMSO- and thiostrepton-treated cells. The classification of gene function through DAVID bioinformatics resources (Huang, Sherman et al. 2007) indicates one of the pathways enriched by these genes is DNA damage and repair pathway. Genes involved in DNA damage and repair, such as *MLH3*, *FANCF*, and *BRCC3* were downregulated while pro-apoptotic genes such as *DDIT4*, *DDIT3*, and *GADD45A* were upregulated (Fang, Madden et al. 2018).

To further characterize the effect of thiostrepton on the expression of FOXM1 target genes, we also analyzed ENCODE dataset and focused on DNA repair genes identified from the FOXM1 ChIP-sequencing. We focused on genes involved in HR repair pathway and analyzed the expression of *FOXM1*, *FANCF*, *BRCA1*, *BRCC3*, *BRIP1*, *NBS1*, *Skp2*, and *Csk1*. In three different cell lines, 5 μM (OVCA420* and OV90) and 2.5 μM (ES-2) thiostrepton treatment resulted in downregulation of the expression of FOXM1 target genes (Figure 2.4.5.A-C). Interestingly, thiostrepton treatment lead to variable upregulated *FOXM1a* and *FOXM1b* in ovarian cancer cells while consistent downregulation of *FOXM1c* and its canonical target gene *CCNB1* was observed in these cells (Figure 2.4.5.D-F).

At protein levels, only BRCA1 and FOXM1 were consistently downregulated following treatment with thiostrepton (Figure 2.4.5.G). To resolve the inconsistency between mRNA and protein expression, we analyzed the stability of these proteins in question. Following the inhibition of protein synthesis by cycloheximide, we observed a decrease in BRCA1 and FOXM1. The half-life of BRCA1 and FOXM1 was less than 60 minutes in OVCA420* (Figure 2.4.5.I-J). In contrast, the half-life for BRCC3 and FANCF was longer than 24 hours (Figure 2.4.5.I-J). Similarly, the half-life for BRCC3 and FANCF was longer than 24 hours in OV90 (Figure 2.4.5.K-L). The longer half-life of BRCC3 and FANCF may explain why a substantial decrease in mRNA does not correspond with a decrease in protein levels. Expression of DNA repair genes including BRCA1 is known to be cell cycle dependent, and changes in expression of these genes may be secondary to cell cycle changes induced following thiostrepton treatment. However, we did not observe a marked change in cell cycle profile at 4 hours, in two different cell lines, when BRCA1 is downregulated by thiostrepton in these cells (Figure 2.4.5.M-N), suggesting that changes of expression of these genes including *BRCA1* were not due to cell cycle arrest. These data suggest

that thiostrepton treatment leads to inhibition of FOXM1 expression and its target HR genes, and thus can induce a *"BRCAness"* like phenotype in ovarian cancer cells.

Figure 2.4.5

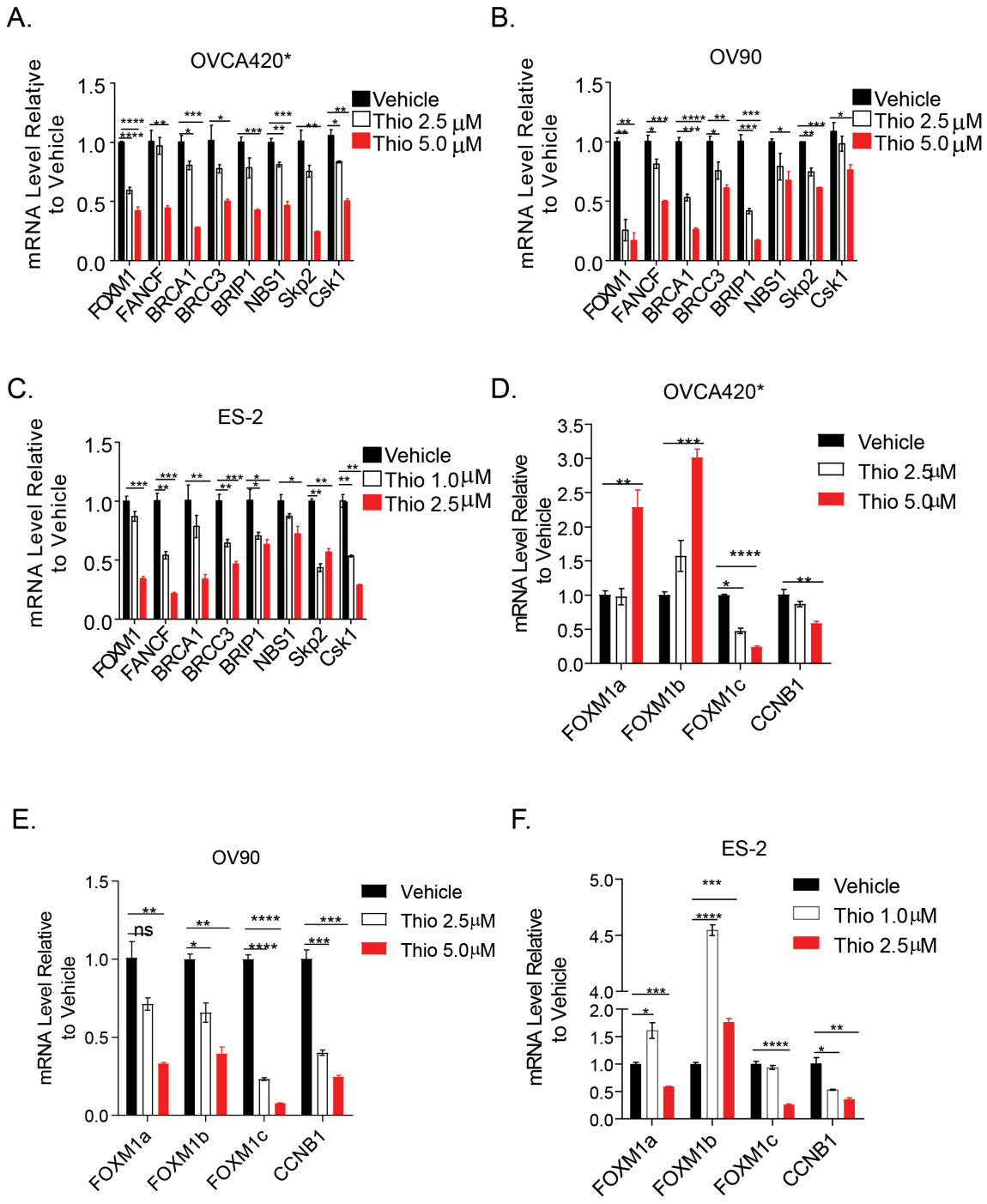
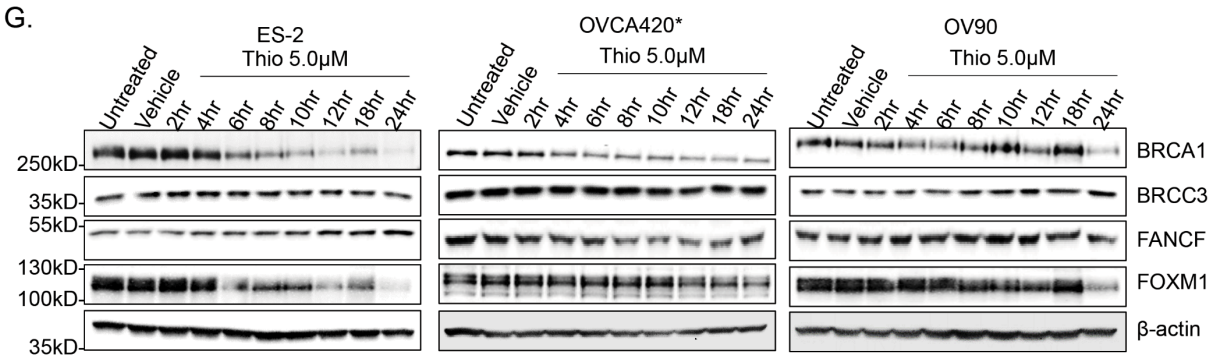
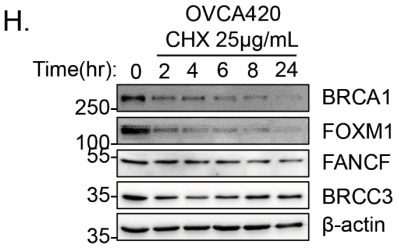
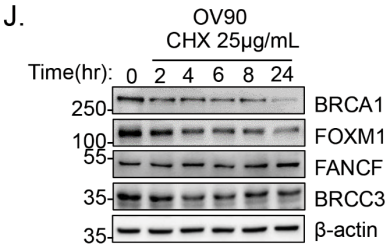
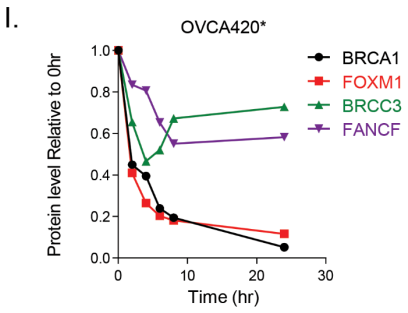


Figure 2.4.5 Cont.


G.
ES-2
Thio 5.0μM
OVCA420*
Thio 5.0μM
OV90
Thio 5.0μM
Untreated
Vehicle
2hr
4hr
6hr
8hr
10hr
12hr
18hr
24hr
250kD
35kD
55kD
130kD
100kD
35kD
BRCA1
BRCC3
FANCF
FOXM1
β-actin


H.
OVCA420
CHX 25μg/mL
Time(hr): 0 2 4 6 8 24
250
100
55
35
35
BRCA1
FOXM1
FANCF
BRCC3
β-actin


J.
OV90
CHX 25μg/mL
Time(hr): 0 2 4 6 8 24
250
100
55
35
35
BRCA1
FOXM1
FANCF
BRCC3
β-actin


I.
OVCA420*
Protein level Relative to 0hr
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
Time (hr)
BRCA1
FOXM1
BRCC3
FANCF

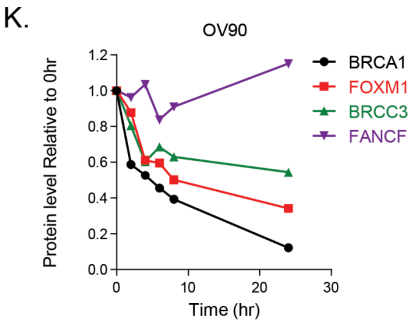

K.
OV90
Protein level Relative to 0hr
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
Time (hr)
BRCA1
FOXM1
BRCC3
FANCF

Figure 2.4.5 Cont.

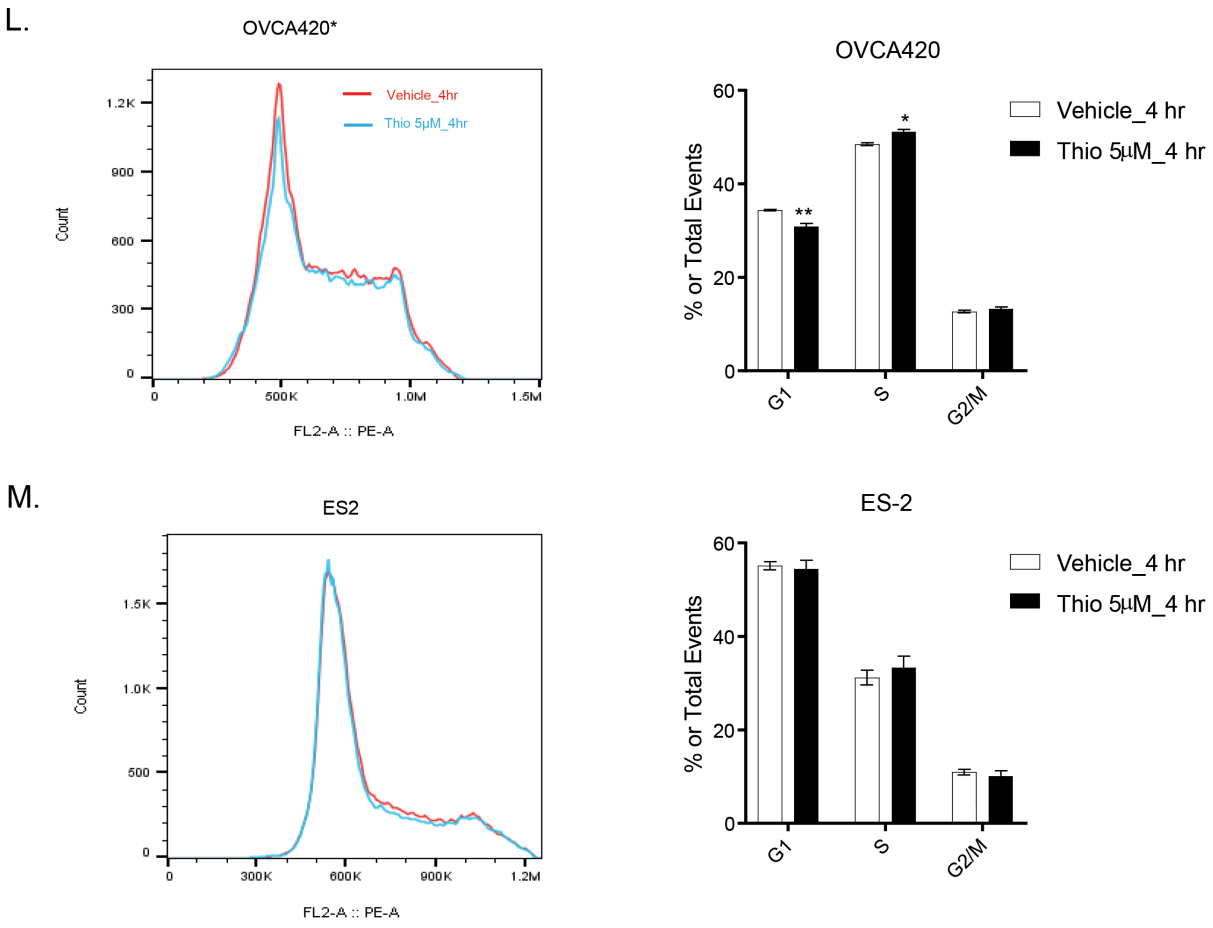


Figure 2.4.5: Thiostrepton downregulates FOXM1 target HR genes. A-C) qRT-PCR analysis to show that treatment of cells with thiostrepton results in downregulation of mRNA expression of *FOXM1* and its target HR genes in A) OVCA420*, B) OV90, and C) ES-2 cells. **D-F)** qRT-PCR analysis to show that thiostrepton treatment leads to downregulation mRNA expression of all three *FOXM1* isoforms and *CCNB1*, which is used as a control target of FOXM1, in all three cell lines. **G)** Western blot analysis to show a change of protein levels for BRCA1, FANCF, and BRCC3 with thiostrepton treatment. ES-2, OVCA420*, and OV90 cells were treated with 5 µM of thiostrepton for 0, 2,4,6,8,10,12,18, or 24 hours. Beta-actin was used as a loading control. **H-K)** Protein half-life analysis with cycloheximide (CHX) shows that FOXM1 and BRCA1 have short half-life, while BRCC3 and FANCF proteins are relatively stable. H) and J) Western blot analysis to check protein levels of FOXM1, BRCA1, FANCF, and RAD51 after 0, 2,

4, 6, 8, or 24 hr treatment of CHX (25μg/mL). I) and K) Quantification of protein levels in OVCA420* and OV90 respectively. **L-M)** Cell cycle analysis with thiostrepton treatment. L) OVCA420* and M) ES-2 cells were treated with vehicle or 5 μM thiostrepton for 4 hrs before PI staining. Cell cycle was analyzed by flow cytometry. Left: Representative cell cycle profiles of vehicle and thiostrepton treated cells. Right: Quantification of cells in different phases. Data are shown as mean ± SEM. *$p<0.05$, **$p<0.01$. One representative experiment with triplicates was shown from three independent experiments.

2.4.6 Thiostrepton induces apoptosis by increase of pro-apoptotic genes and decrease of anti-apoptotic genes

In addition to genes involved in DNA repair pathway, the analysis of microarray dataset from the Connectivity Map project also indicated that thiostrepton affects the expression of genes involved in apoptosis. Therefore, we sought to confirm the expression of pro- and anti-apoptotic genes affected by exposure to thiostrepton. QRT-PCR analysis indicated that thiostrepton treatment leads to upregulated *DDIT3*, *GADD45A* while the anti-apoptotic gene *BCL-2* was downregulated (Figure 2.4.6.A-B). Consistent with these results, exposure to thiostrepton can induce PARP1 and caspase 3 cleavages in two different cell lines (Figure 2.4.6.C-D). Finally, results from the caspase 3 activity assay demonstrated higher caspase 3 activity in cells treated with thiostrepton alone or in combination with olaparib (Figure 2.4.6.E-F).

Figure 2.4.6

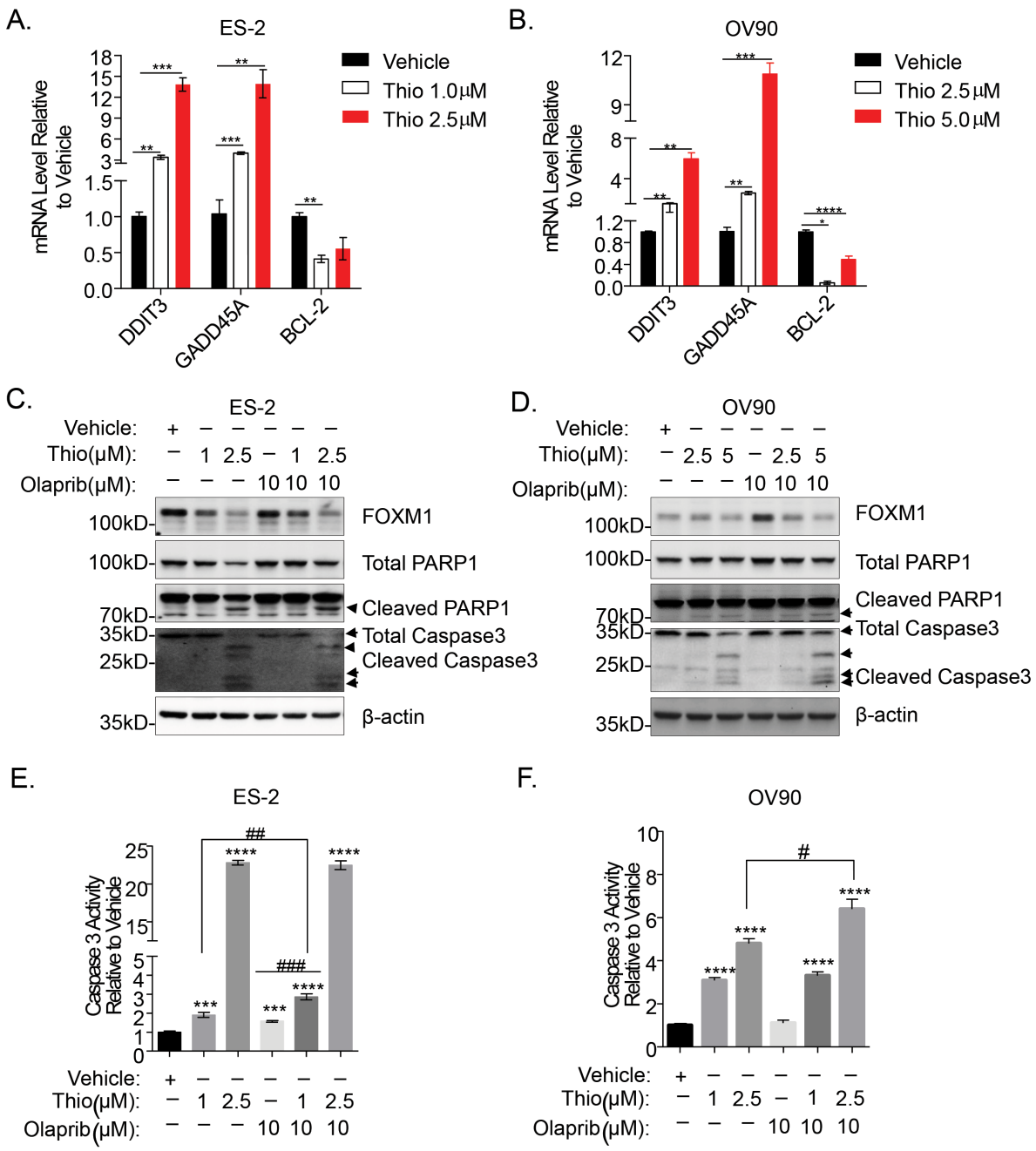


Figure 2.4.6: Thiostrepton treatment induces apoptosis by increasing the expression of pro-apoptotic genes and decreasing the expression of anti-apoptotic genes. A-B) qRT-PCR analysis to measure mRNA levels of pro-apoptotic genes *DDIT3*, *GADD45A* and anti-apoptotic gene *BCL-2*. A) ES-2 cells were treated with vehicle, 1 μM or 2.5 μM thiostrepton, B) OV90 cells were treated with vehicle, 2.5 μM or 5 μM

thiostrepton for 24 hours. Results represent experiments performed in triplicates. **C-D)** Western blot analysis shows that thiostrepton increases PARP1 and Caspase 3 cleavage with and without olaparib in ES-2 and OV90 cells. C) ES-2 and D) OV90 cells were treated with thiostrepton, olaparib or thiostrepton & olaparib for 30 hours. Beta-actin serves as loading control. **E-F)** Exposure to thiostrepton results in increased caspase 3 activity. Caspase 3 activity assay was performed 30 hours after drug treatment in E) ES-2 and F) OV90 cells. Data shown as mean ± SEM. Statistics was done with Student's t-test, #$p<0.05$, ##$p<0.01$, ###&***$p<0.001$, ****$p<0.0001$. Results represent experiments performed in duplicates.

2.4.7 Thiostrepton enhances DNA damage and PARP1 trapping in cells treated with olaparib

Because thiostrepton treatment can lead to downregulation of FOXM1 and its target genes involved in DNA repair pathway, we also tested the extent to which thiostrepton can enhance DNA damage in cells treated with olaparib. The results from comet assay indicated that thiostrepton treatment alone increases DNA damage (Figure 2.4.7.A). In addition, higher levels of DNA in comet tail were observed in cells treated with 10 μM olaparib and thiostrepton. The increase in DNA damage following thiostrepton treatment could be explained in part by the downregulation of FOXM1 because *FOXM1* knockdown by siRNA produces a similar increase in DNA damage (Figure 2.4.7.B). Meanwhile, increased level of phospho-H2AX (γH2AX) was seen with thiostrepton treatment and the increase was enhanced when combined with olaparib (Figure 2.4.7.C-D). These results were consistent with a critical role of FOXM1 in regulating DNA repair genes, and a decreased expression of FOXM1 may compromise DNA repair function by downregulating several target genes involved in DNA repair. Consistent with an increase in DNA damage following FOXM1 inhibition, we also observed an increase in trapped PARP1 in cells treated with either 5 or 10 μM thiostrepton, or 40 μM olaparib (Figure 2.4.7.E). This observation provides an additional mechanism to explain the enhanced sensitivity to olaparib in ovarian cancer cells since PARP trapping onto chromatin after PARP inhibitor treatment is considered to be more cytotoxic than inhibition of enzymatic activity of PARP (Murai, Huang et al. 2012).

Figure 2.4.7

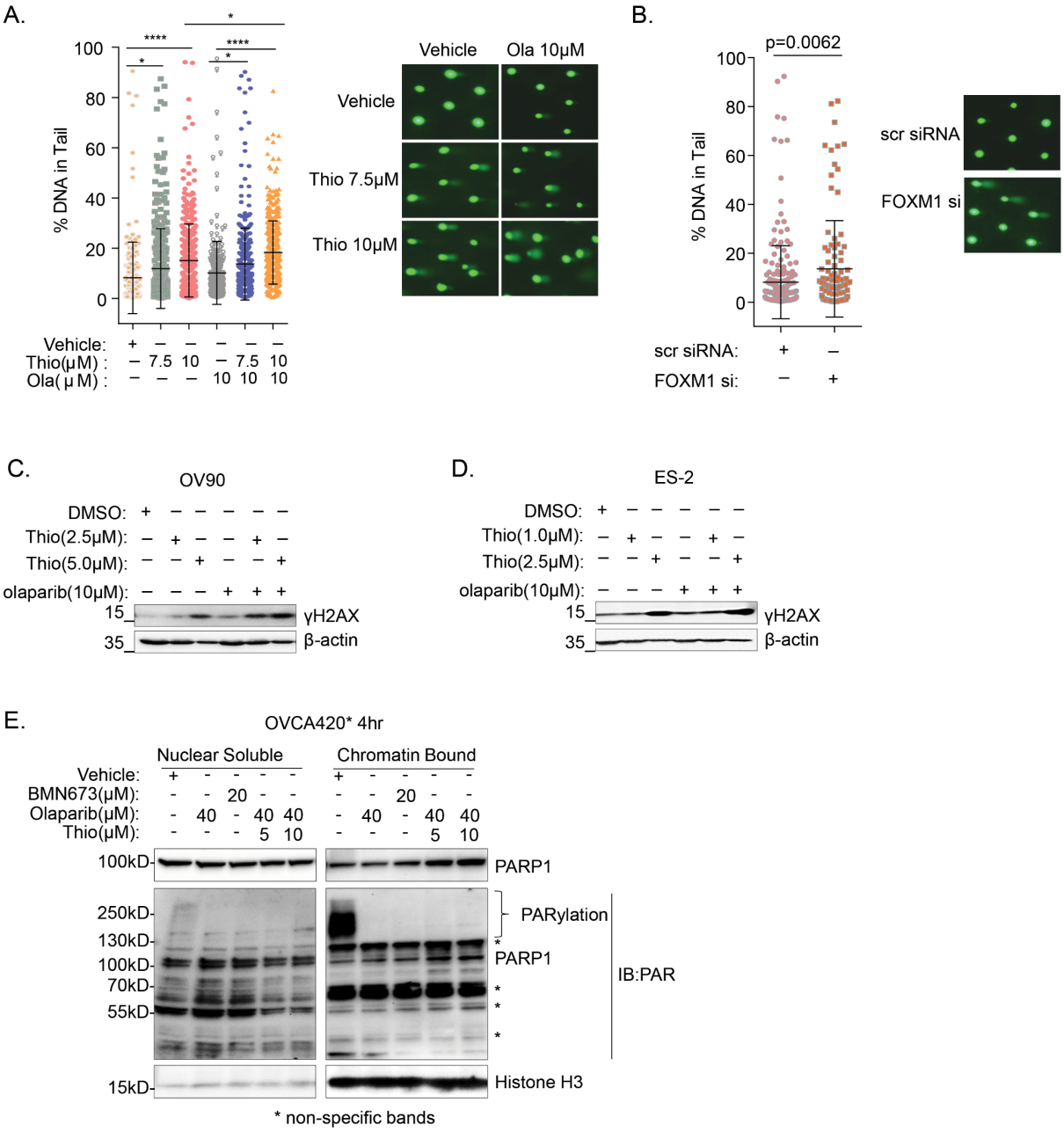


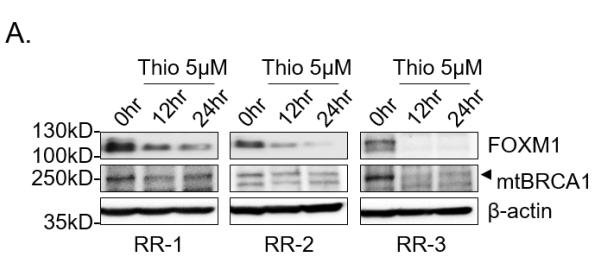
Figure 2.4.7: Thiostrepton enhances DNA damage and PARP1 trapping in cells co-treated with olaparib. A) Comet assay shows that thiostrepton treatment can enhance DNA damage in cells treated with olaparib. OVCA420* cells were pretreated with vehicle or thiostrepton for 4 hours in 6-well plates before seeding onto CometChip (Trevigen). Then cells were treated with vehicle, 7.5 μM or 10 μM thiostrepton

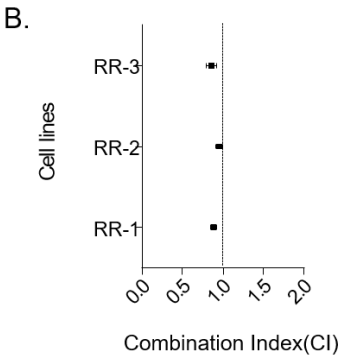
and olaparib (10 μM) for another 4 hrs. Comets were analyzed with Trevigen Comet Analysis Software after imaging under a 4X fluorescent microscope. Data were shown as percent of DNA in comet tail. Representative comet images were shown on the right. The significance analysis was performed with One-way ANOVA. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001. Right: Representative images of comets. Results represent experiments performed in triplicates. **B)** Comet assay shows that FOXM1 siRNA knockdown leads to increase of DNA damage. Comet assay was performed 72 hours after siRNA transfection. **C-D)** Western blot analysis shows that thiostrepton increases γH2AX levels. C) OV90 or D) ES-2 cells were treated with the indicated drug(s) for 48 hours. Beta-actin serves as a loading control. **E)** PARP trapping analysis shows that thiostrepton enhanced PARP1 trapping to chromatin in the presence of olaparib. OVCA420* cells were treated with the indicated drug(s) for 4 hours and lysed to separate nuclear soluble and chromatin-bound fraction. First blotted with PARP1 antibody and secondly with Poly (Ribose-ADP) antibody. * represents non-specific band detected by PAR antibody. Results were from a representative of two independent experiments.

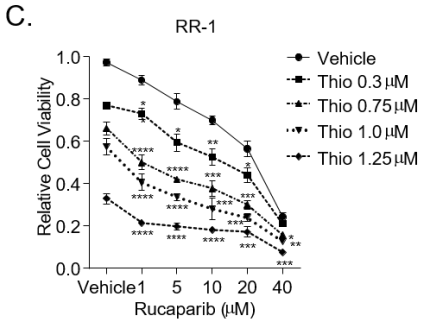
2.4.8 Thiostrepton sensitizes resistant cells to rucaparib by downregulating stabilized mutant BRCA1

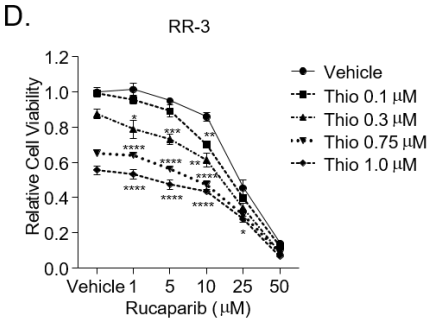
Previous studies reported an enhanced protein level of mutant BRCA1 as an epigenetic mechanism associated with acquired resistance to rucaparib (Johnson, Johnson et al. 2013). Although the parental breast cancer cell line, MDA-MB-436, are sensitive to rucaparib due to the presence of a mutated *BRCA1*, the resistant clones derived from these cells expressed high levels of mutant BRCA1 that partially restore BRCA1 mediated HR function and confer resistance to rucaparib. We used these resistant clones to test the extent to which treatment with thiostrepton could restore sensitivity to rucaparib in these cells. Thiostrepton treatment lead to downregulation of the expression of FOXM1 as well as mutant BRCA1 (Figure 2.4.8.A). Consistent with this downregulation, we observed an increase in sensitivity to rucaparib in thiostrepton-treated cells (Figure 2.4.8.B-D). In addition, mild synergistic interactions between thiostrepton and rucaparib were observed at different combinations. Clonogenic assays further corroborated these synergistic interactions between the two drugs (Figure 2.4.8.E-G). This enhanced sensitivity to rucaparib was also observed in resistant cells following the *FOXM1* knockdown by siRNA (Figure 2.4.8.H-J), indicating that enhanced sensitivity to rucaparib by thiostrepton could be explained in part by its effect on FOXM1 expression. These data suggest that inhibition of FOXM1 associated with thiostrepton treatment can be an effective way to overcome acquired resistance to PARP inhibitors.

Figure 2.4.8







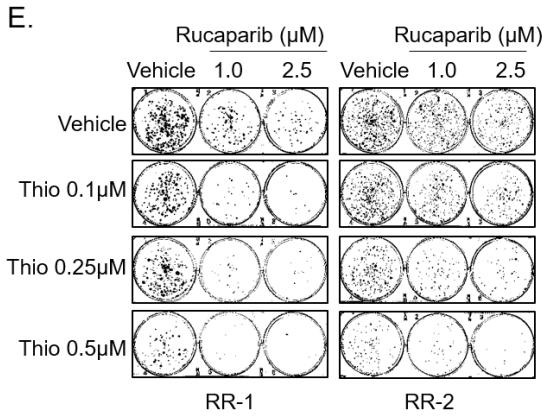


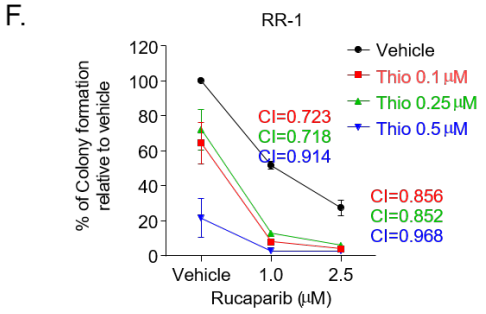
Combination Index			
	Thio 0.3μM	Thio 0.75μM	Thio 1.0μM
Ruc 5μM	1.163	0.841	0.891
Ruc 10μM	1.025	0.862	0.867
Ruc 20μM	0.989	0.886	0.88

RR-1

Combination Index			
	Thio 0.3μM	Thio 0.75μM	Thio 1.0μM
Ruc 5μM	0.659	0.868	0.897
Ruc 10μM	0.642	0.832	0.921
Ruc 20μM	0.913	1.005	1.029

RR-3





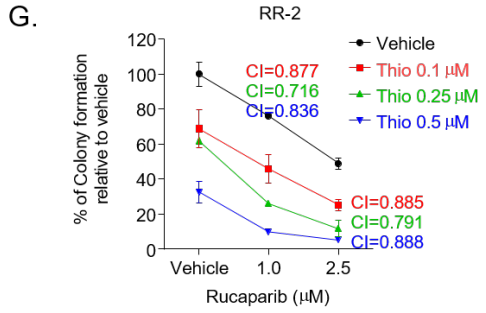


Figure 2.4.8 Cont.

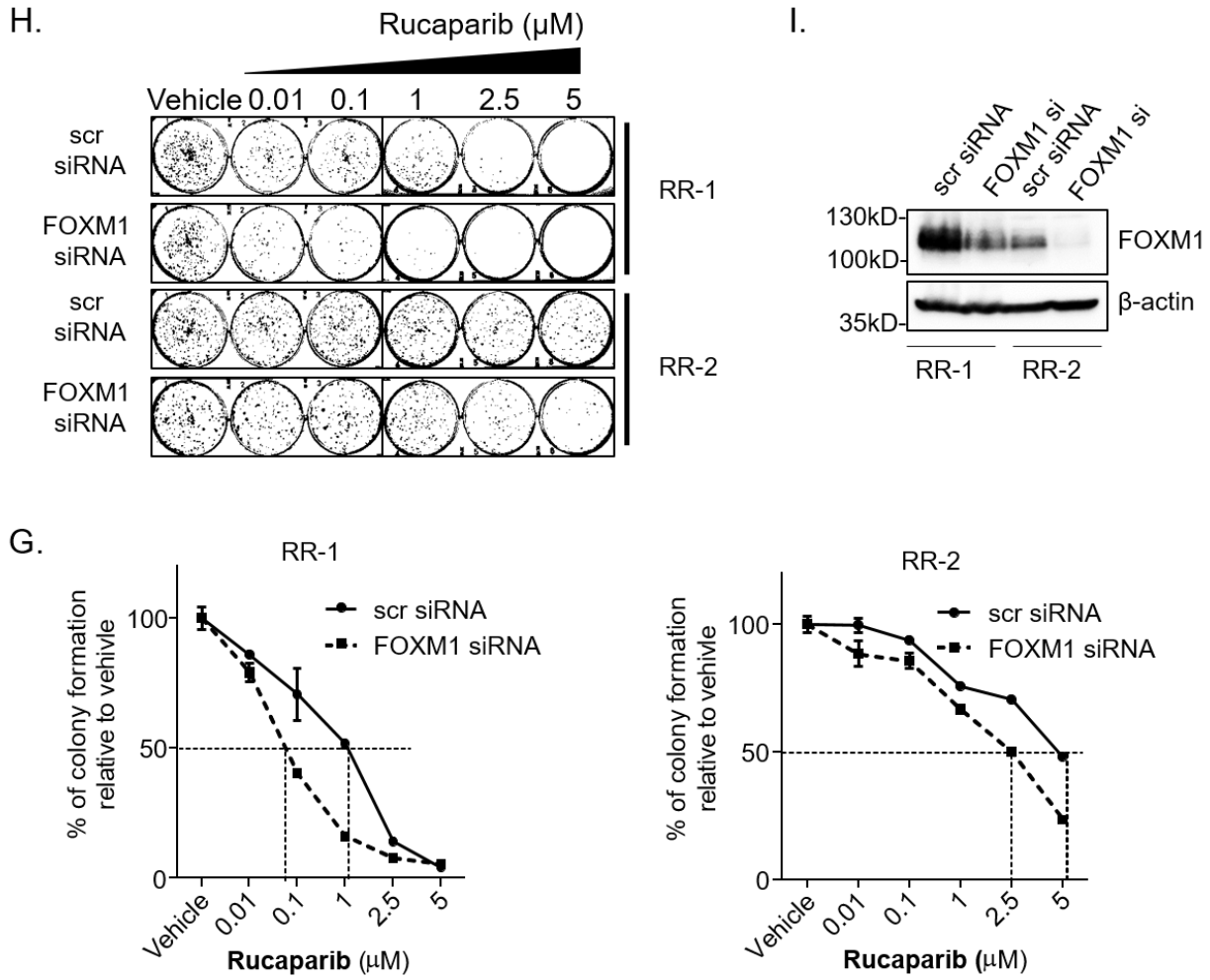


Figure 2.4.8: Thiostrepton treatment can sensitizes resistant cells to rucaparib by downregulating FOXM1 and stabilized mutant BRCA1 (mtBRCA1). A) Western blot analysis to show downregulation of FOXM1 and mtBRCA1 in three rucaparib resistant MDA-MB-436 cells, RR-1, RR-2, and RR-3. Cells were treated with 5 μM thiostrepton for 0, 12 or 24 hours respectively. Beta-actin serves as a loading control. **B)** Mild synergistic interactions between thiostrepton and rucaparib by SRB assay. CIs for different combinations were calculated and CIs resulted from drug effects between 0.2-0.8 were used in the plot. Data are shown as mean with 95% confidence intervals. **C-D)** Thiostrepton sensitizes rucaparib resistant cells to rucaparib. Upper: Line-graphs show cell viability relative to the vehicle for C) RR-1 and D) RR-3.

Data shown as analyzed using the Student's t-test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Bottom: Calculated CIs for different combinations of thiostrepton and rucaparib. Results represent experiments with four replicates (B-D). **E-G)** Clonogenic assay shows moderate synergistic effects of thiostrepton and rucaparib. Cells were exposed to the drug(s) for 3 days followed by around 3 weeks' no drug incubation to allow colony formation. E) Representative images of colonies in 6 well plates. F-G) Quantification of colonies formed for each treatment. Line graphs show the percent of colony formation relative to the vehicle. Data are shown as mean ± SEM. CIs for different drug combinations were calculated and indicated. Results represent experiments performed in duplicates (E-G).

2.5 Discussion

In this study, FOXM1 was identified as a component of the adaptive cellular pathway response to olaparib. Olaparib treatment resulted in induced expression of FOXM1 and activation of the FOXM1 pathway as evidenced by the enhanced nuclear localization of FOXM1 and the increased binding of FOXM1 to its target genes. The disruption of FOXM1 pathway either by RNAi or chemical inhibition with thiostrepton decreased the levels of FOXM1 expression and sensitized the cancer cells to olaparib and carboplatin. Furthermore, thiostrepton decreased the levels of DNA repair genes involved in HR pathway and induces *"BRCAness"* in ovarian cancer cells, thereby resensitizing the rucaparib-resistant breast cancer cells to rucaparib. At the same time of this study, Tassi et al., reported that FOM1 expression is significantly associated with chemotherapy resistance, and that *FOXM1* knockdown enhances the cytotoxic effects of chemotherapeutic agents including olaparib in non-serous epithelial cancer cells (Tassi, Todeschini et al. 2017) which is consistent with the observations I described in this chapter.

Previous studies indicate that FOXM1 regulates the expression of *BRIP1* and DNA damage repair pathway following epirubicin treatment (Monteiro, Khongkow et al. 2013). In addition, a spontaneous increase in γH2AX foci has been reported in cancer cells following the depletion of FOXM1 by RNAi (Tan, Raychaudhuri et al. 2007). Consistent with these results, this study provided additional supporting evidence that *FOXM1* knockdown and thiostrepton treatment results in increased γH2AX level, enhanced DNA damage, and PARP1 trapping to the damaged DNA in the presence of olaparib. Olaparib has been shown to produce moderate levels of PARP trapping (Murai, Huang et al. 2012). The increase of PARP trapping in thiostrepton and olaparib treated cells may be a result of increased DNA damage associated with thiostrepton exposure, which leads to the recruitment of DNA repair machinery to the damage sites, including PARP1

which is responsible for the base excision repair (BER). A significant increase in γH2AX foci either by RNAi or thiostrepton underlies the important role FOXM1 plays in regulating DNA repair genes and maintaining DNA repair efficacy (Zona, Bella et al. 2014). Accordingly, FOXM1 is described as an "emerging master regulator of DNA damage response" (Zona, Bella et al. 2014). Therefore, my results indicating that FOXM1 is induced in cells following treatment with olaparib and that its enhanced expression regulates the adaptive cellular response to DNA damage signal provide an important biological basis for targeting FOXM1 pathway to overcome resistance to olaparib and carboplatin.

It is important to note that previous studies have also shown that FOXM1 is stabilized by DNA damaging agents, such as ionizing radiation, etoposide and UV (Tan, Raychaudhuri et al. 2007, Teh, Gemenetzidis et al. 2010). Tan *et al.* reported that FOXM1 is phosphorylated by Chk2 at S361, leading to the stability of FOXM1 (Tan, Raychaudhuri et al. 2007). They reported that FOXM1 regulates BRCA2 and XRCC1. In addition, Monteiro *et al.* reported that FOXM1 is required for HR pathway but indispensable for NHEJ (Monteiro, Khongkow et al. 2013). Finally, Zhang *et al.* reported that FOXM1 regulates RAD51 expression (Zhang, Wu et al. 2012). Consistent with these results, we observed FOXM1 occupancy to DNA repair genes, such as *BRCA1*, *RAD51*, *FANCF*, *RAD51D*, and *FANCD2*, in response to olaparib. In addition, inhibition of FOXM1 via thiostrepton treatment decreased the expression of DNA repair genes, such as *BRCA1* and *BRCA2*, thereby disrupting an adaptive response mediated by FOXM1.

Considering that adaptive responses to chemotherapy provide transient resistance to chemotherapy and may facilitate the eventual acquisition of more stable traits associated with chemotherapy resistance, our study provides an important insight into a potential role of the FOXM1 pathway in the adaptive response to olaparib. Our studies also suggest the potential use

of thiostrepton as a chemotherapeutic agent to disrupt FOXM1 pathway and to enhance sensitivity to olaparib. Although thiostrepton was initially isolated from *Streptomyces azureus* and used as a topical antibiotic (Kutscher, Zegarelli et al. 1959), its current use in human is limited by the low solubility and bioavailability. However, recent studies have shown that micelle-formulated thiostrepton has better solubility and pharmacodynamic effect on the tumor in xenograft models (Wang and Gartel 2011). Also, several drug repurposing studies have tested the efficacy of thiostrepton in tumor xenograft mouse models, including ovarian cancer, leukemia and laryngeal squamous cell carcinoma (Zhang, Cheng et al. 2014, Buchner, Park et al. 2015, Jiang, Wu et al. 2015). In addition to thiostrepton, siomycin A is another cyclic oligopeptide isolated from *Streptomyces sioyanesis* and shows inhibitory effects on FOXM1 transcriptional activity and expression (Radhakrishnan, Bhat et al. 2006). Finally, another inhibitor FDI-6 is a small molecule FOXM1 inhibitor that blocks FOXM1 binding to DNA (Gormally, Dexheimer et al. 2014). Although micro-molar concentrations of FDI-6 is required to inhibit FOXM1, further development of this compound may result in therapeutics that can effectively target FOXM1 and enhance sensitivity to existing chemotherapeutics, such as olaparib and carboplatin.

Data from this study showed that FOXM1 plays an important role in an adaptive response to PARP inhibitors. *FOXM1* expression levels inversely correlated with olaparib sensitivity, and knockdown of *FOXM1* sensitized ovarian cancer cells to olaparib and decreased the number of persisting cells that can produce colonies. The FOXM1 inhibitor, thiostrepton, induces a *"BRCAness"* like phenotype by downregulating homologous recombination repair genes, which in turn results in increased apoptosis, and enhanced therapeutic effects of olaparib and carboplatin in ovarian cancer cells. In addition, thiostrepton treatment overcome resistance to rucaparib by downregulating FOXM1 and stabilized mutant BRCA1 in resistant breast cancer cells. Inhibition

of FOXM1 represents an effective strategy to overcome PARP inhibitor resistance and that disrupting FOXM1-mediated adaptive pathway may produce better therapeutic effects for PARP inhibitors.

Chapter 3. Targeting FOXM1-MYC nexus with BET inhibitor enhances therapeutic effects of PARP inhibitor in ovarian cancer

3.1 Abstract

FOXM1 and *MYC* are overexpressed in epithelial ovarian cancer (Cancer Genome Atlas Research 2011, Ross, Ali et al. 2013). FOXM1 regulates the expression of HR genes and modulates PARP inhibitor sensitivity. However, the potential role of c-MYC in PARP inhibitor sensitivity is not yet fully characterized. In this study, we found that expression of specific target genes of FOXM1 in HR pathway, *BRCA1*, *BRCA2*, *FANCF* and *BRIP1*, were not affected after the FOXM1 knockdown by siRNAs. Interestingly, we observed an increase of c-MYC after FOXM1 knockdown. ENCODE data analysis revealed that both FOXM1 and c-MYC bind to a subset of HR target genes, suggesting a potential co-regulation of these genes by FOXM1 and c-MYC. In addition, TCGA data analysis showed that *FOXM1, MYC,* and *BRD4* are highly expressed in ovarian cancer. Considering that BET inhibitors inhibit BRD4 and downregulate both FOXM1 and c-MYC, we investigated the effect of BET inhibitors on PARP inhibitor sensitivity in ovarian cancer cells. In three cancer cell lines with a lower sensitivity to PARP inhibitor, BET inhibitor, (+)-JQ1, attenuated the upregulation of c-MYC after FOXM1 knockdown and decreased the expression of FOXM1, *BRCA1* and *RAD51* genes. ChIP-qPCR demonstrated decreased binding of FOXM1 and c-MYC to *BRCA1* and *RAD51* promoters, suggesting that the decreased expression of *BRCA1* and *RAD51* can be attributable to the suppression of FOXM1 and *MYC*. Cell viability assays showed (+)-JQ1 decreased cell viability in a dose-dependent manner, and enhanced the cytotoxic effects of PARP inhibitor olaparib in these cells. Strikingly, multiple combinations at the sub-lethal doses of (+)-JQ1 and olaparib completely suppressed colony formation. Similarly, (+)-JQ1 also enhanced the cytotoxic effect of rucaparib in acquired resistance cells derived from MDA-MB-436 breast cancer cells. We showed that (+)-JQ1 treatment leads to downregulation of FOXM1, RAD51, and mutant BRCA1, which was originally stabilized to confer rucaparib

resistance in the resistant cells. Taken together, these data suggest that targeting the FOXM1-MYC nexus with BET inhibitor, (+)-JQ1, induces of *"BRCAness"* like phenotype by downregulating *BRCA1* and *RAD51* and in turn restores the sensitivity to PARP inhibitors in cancer cells with primary and acquired resistance to PARP inhibitor.

3.2 Introduction

Cancer cells with deleterious *BRCA1/2* mutations are hypersensitive to PARP inhibitor (Bryant, Schultz et al. 2005, Farmer, McCabe et al. 2005). This observation was later extended to other cancer cells with a broader deficiency in HR pathway (McCabe, Turner et al. 2006, Mendes-Pereira, Martin et al. 2009, Drew, Mulligan et al. 2011). The extended clinical activity of PARP inhibitors is due to *"BRCAness"* or HR-deficient (HRD) phenotypes resulting from defects in HR pathway that phenocopy the sensitivity to PARP inhibitors that is similar to *BRCA*-mutated tumors. In high-grade serous ovarian cancer, approximately 50% of cases have HR defects (Cancer Genome Atlas Research 2011), which can result from either direct alterations of HR components, such as *BRCA1/2* (Farmer, McCabe et al. 2005, Veeck, Ropero et al. 2010), or indirect alterations, such as *EMSY* amplification (Hughes-Davies, Huntsman et al. 2003). Ovarian cancer patients were among the first individuals to be evaluated for efficacy using PARP inhibitor (Fong, Boss et al. 2009, Audeh, Carmichael et al. 2010, Tutt, Robson et al. 2010, Ledermann, Harter et al. 2012, Ledermann, Harter et al. 2014). The activity of PARP inhibitors was seen preferentially in ovarian cancer patients possessing a *BRCA* mutant tumor, leading to approval of PARP inhibitors as monotherapy and maintenance therapy for pre-treated ovarian cancer patients with mutated *BRCA1/2* (either germline or somatic). Besides ovarian cancer, PARP inhibitors have shown activities in other cancer types, including breast cancer, prostate cancer, non-small cell lung cancer, and melanoma, and are being evaluated in clinical trials (Plummer, Lorigan et al. 2013, Bhalla and Saif 2014, Levra, Olaussen et al. 2014, Livraghi and Garber 2015, Ramakrishnan Geethakumari, Schiewer et al. 2017).

A clear survival advantage with PARP inhibitor was seen in patients with *BRCA* mutations and patients who are responsive to platinum-based chemotherapy. Unfortunately, the response rate

in patients with no *BRCA* mutations and those with platinum resistance disease is much less (Fong, Boss et al. 2009, Audeh, Carmichael et al. 2010, Mirza, Monk et al. 2016). This selective activity largely limits the broader application of PARP inhibitors in cancer treatment. Meanwhile, like chemotherapy, resistance to PARP inhibitor eventually develops in patients (Fojo and Bates 2013). One of the major causes of PARP inhibitor (PARPi) resistance in the clinic is the restoration of HR proficiency (Lord and Ashworth 2013). Drug combinations have been designed and evaluated in preclinical and early clinical trials to extend the therapeutic effect of PARP inhibitors and/or to overcome PARPi resistance (Benafif and Hall 2015). These combinations were mostly designed with the goal of enhancing the cytotoxic effects, including PARPi in combination with ionizing radiation or chemotherapeutic agents (Miura, Sakata et al. 2012, Al-Ejeh, Shi et al. 2013, Plummer, Lorigan et al. 2013), such as topotecan, dacarbazine, and temozolomide (Evers, Drost et al. 2008, Zander, Kersbergen et al. 2010, Norris, Adamson et al. 2014). Combination of PARPi with other agents, such as VEGF (Liu, Barry et al. 2014), PI3K (Wang, Li et al. 2016, Wang, Wang et al. 2016), TGFβ (Liu, Zhou et al. 2014), and immune checkpoint inhibitors (Gadducci and Guerrieri 2017) have also been explored. Even though preclinical studies have shown promising therapeutic effects with these combinations, results from clinical trials were disappointing. The dose-limiting severe hematological adverse effects were observed in the combination of olaparib with topoisomerase I inhibitor, Topotecan (Samol, Ranson et al. 2012), or with a potent VEGF inhibitor, Cediranib (Liu, Barry et al. 2014). Therefore, there is an immediate need to explore new rational drug combinations that maximize clinical benefits and minimize adverse effects. These rational combinations must focus on compromising HR proficiency and restoring or enhancing PARP inhibitor sensitivity in cancer cells.

In Chapter 2, I showed that FOXM1 positively regulates DNA repair genes, especially HR genes, and inhibition of FOXM1 as a result of thiostrepton exposure disrupts the FOXM1 transcriptional network and enhances the sensitivity to PARP inhibitors in PARPi-resistant cancer cells. Although the *in vivo* efficacy of thiostrepton has been shown by several groups (Wang and Gartel 2011, Zhang, Cheng et al. 2014, Buchner, Park et al. 2015, Jiang, Wu et al. 2015), further studies are needed to validate its clinical activity. FOXM1 inhibition is only one of documented activities of thiostrepton, and it was shown to function as a proteasome inhibitor as well (Aminake, Schoof et al. 2011, Gartel 2011). In this regard, a more specific targeted drug may be desirable to combine with olaparib. Our data show that FOXM1 downregulation with siRNA is not sufficient to suppress the expression of downstream HR genes, such as *BRCA1*, *BRCA2*, *FANCF* and *BRIP1*. Meanwhile, c-MYC was upregulated after FOXM1 silencing via RNAi approaches. We suspect that c-MYC upregulation served as a compensation mechanism to regulate HR genes after FOXM1 knockdown, and therefore we propose that targeting both FOXM1 and c-MYC may provoke better impairment of HR.

BET inhibitors are a class of drugs that were designed to reversibly bind the bromodomains of BET (bromodomain and extra-terminal motif) proteins (BRD2, BRD3, BRD4, and BRDT) to prevent their interactions with acetylated histones and transcription factors (Filippakopoulos, Qi et al. 2010, Nicodeme, Jeffrey et al. 2010, Loven, Hoke et al. 2013) to decrease the transcription of target genes. Since BRD4 was shown to drive pathogenesis in various types of cancers (Shi and Vakoc 2014), BET inhibitors have been studied in cancers (French, Ramirez et al. 2008, Da Costa, Agathanggelou et al. 2013, Li, Guo et al. 2016, McCleland, Mesh et al. 2016, Shu, Lin et al. 2016). Importantly, BET inhibitors have been shown to block the expression of c-MYC (Delmore, Issa et al. 2011, Sun and Gao 2017), which was long considered undruggable oncogenic transcription

factor (Koh, Sabo et al. 2016). In addition, *in vitro* and preclinical studies show that BET inhibitors are active in overcoming resistance to other targeted therapies when used in combination therapies (Knoechel, Roderick et al. 2014, Korkut, Wang et al. 2015). Interestingly, Zhang et al. show that BET inhibitor downregulates FOXM1 pathway, but the molecular mechanisms associated with FOXM1 downregulation by BET inhibitors are not fully investigated (Zhang, Ma et al. 2016).

In this chapter, we tested the effects of the combination of BET inhibitor (+)-JQ1 with olaparib or rucaparib in primary and acquired resistance cancer cells and showed that (+)-JQ1 inhibits the expression of both FOXM1 and *MYC*. Consequently, their target genes in HR pathway, such as *BRCA1* and *RAD51* are downregulated, and the resulting HR impairment in these cancer cells sensitizes them to PARP inhibitors.

3.3 Materials and Methods

Cell lines and cell culture

ES-2 cell was maintained in MCDB105 and M199 (1:1) (Sigma, USA) containing 5% FBS (Sigma). OV90 cells were maintained in MCDB105 and M199 (1:1) with 15% FBS. OVCA420* cells were cultured in DMEM (Sigma and Caisson Labs, USA) supplemented with 10% FBS. MDA-MB-436 and its derivative rucaparib resistant cells RR-1, RR-2 were generated by Dr. Neil Johnson laboratory at Fox Chase Cancer Center (Johnson, Johnson et al. 2013) and were maintained in RPMI1640 (Sigma and Caisson Labs) supplemented with 10% FBS. All the media were supplemented with 100 units/mL penicillin and 100 µg/mL streptomycin.

Antibodies and Compounds

Rabbit polyclonal anti-FOXM1 antibody (C-20, sc-502) and rabbit polyclonal anti-BRCA1 antibody (C-20, sc-642) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal anti-RAD51 antibody (5B3/6, GTX23638) was from GeneTex (Irvine, CA, USA). Rabbit monoclonal anti-c-MYC antibody (Y69) was purchased from Abcam (Cambridge, MA, USA). Mouse monoclonal anti-beta actin antibody (A1978) was from Sigma-Aldrich (St Louis, MO, USA). For secondary antibodies, horse anti-mouse IgG-HRP antibody (7076S) was purchased from Cell Signaling Technologies, goat anti-rabbit IgG-HRP antibody (sc-2030) was obtained from Santa Cruz Biotechnology. Olaparib (AZD2281, Ku-0059436) and rucaparib was purchased from Selleckchem (TX, USA). Stock solutions were made with DMSO at 50 mM and stored at -80 °C. ZBC260 was provided by Dr. Shaomeng Wang from the University of Michigan. The stock solution was made with DMSO in 10 mM and aliquot to store at -80°C. (+)-JQ1 was

kindly provided by Bradner lab in Dana-Farber Cancer Institute. The stock solution was made in DMSO at 50 mM aliquots and stored at -80 °C.

Immunoblotting

Cells were washed at least twice with PBS at the end of treatments if applicable and then lysed with an appropriate volume of 1X electrophoresis sample buffer (Bio-Rad Laboratories, CA, USA) with 5% β-mercaptoethanol (Sigma-Aldrich). The cell lysates were heated to 95 °C for 5 minutes before using. Immunoblotting procedures were performed as previously described (Zhang, Cheng et al. 2014). For apoptosis markers, cells were collected at the end of treatments, and total proteins were extracted using RIPA buffer (1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS in 1 X PBS) containing protease/phosphatase inhibitor cocktail (Roche). BCA protein assay reagent kit (Pierce) was used to determine protein concentrations. Equal amounts of total proteins were loaded for SDS-PAGE and transferred onto PVDF membranes (GE healthcare).

Chromatin Immunoprecipitation (ChIP)-qPCR

Chromatin Immunoprecipitation was carried out as described before (Johnson, Mortazavi et al. 2007). Briefly, after 20 μM olaparib treatment for 12 hours or 24 hours, cells were cross-linked with 1% formaldehyde (Electron Microscopy Sciences, USA) for 10 min and quenched by cross-linking with excess glycine. The chromatin was sonicated with a Bioruptor Twin (Diagenode) at maximum setting for 12 min (30 s on, 30 s off). The sonicated chromatin was incubated with 1.0 μg FOXM1 antibody (C-20, sc-502, Santa Cruz Technology, USA) at 4 °C for 2-4 hours before purification with 100 μL Protein A/G magnetic beads (88803, Pierce). The beads were washed five times with LiCl wash buffer (100 mM Tris pH7.5, 500 mM LiCl, 1% NP-40, 1% sodium deoxycholate), one time with TE buffer (10 mM Tris-HCl pH7.5 and 0.1 mM Na2EDTA), and

were then eluted with Elution Buffer (1% SDS and 0.1 M NaHCO3). After reverse-crosslinking, the DNA was purified with the QIAQuick PCR cleanup kit (QIAGEN) and used for qPCR, which was performed in a CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad) using RT² SYBR Green qPCR Master Mix (QIAGEN). Primer sequences are listed in Table 3.3.1.

Table 3.3.1

Table 3.3.1

Amplified target	Forward	Reverse
FOXM1(total)	5′AGAATTGTCACCTGGAGCAG	5′TTCCTCTCAGTGCTGTTGATG
FANCF	5’GCATTTGGGTTGGAACTGAG	5’CTTCAAAATCTCCATCCTGCG
BRCA1	5’TAATGCTATGCAGAAAATCTTAGAG	5’TACTTTCTTGTAGGCTCCTTTTGG
BRCA2	5’TTCATGGAGCAGAACTGGTG	5’AGGAAAAGGTCTAGGGTCAGG
BRIP1	5’GCTTAGCCTTACTTTGTTCTGC	5’TTTCACTTACGCCCTCATCTG
18S rRNA	5’GCCCGAAGCGTTTACTTTGA	5’TCCATTATTCCTAGCTGCGGTATC
GAPDH	5’GAAACTGTGGCGTGATGGC	5’CACCACTGACACGTTGGCAG
CCNB1 FOXM1 BS	5’ CGCGATCGCCCTGGAAACGCA	5’CCCAGCAGAAACCAACAGCCGT
BRCA1 FOXM1 BS	5’CAAGGTACAATCAGAGGATGGG	5’TCCTCTTCCGTCTCTTTCCT
RAD51 FOXM1 BS	5’ACCAGGCAGAGAATCTTGTTC	5’TTCAAGTCTAACCCAGTGCAG
RAD51 MYC BS	5'GCTCCATTTCCCACTTCTATCC	5'CTTTTGGCACTTCTGGTCG
BRCA1 MYC BS	5’ CTTCCAGTTGCGGCTTATTG	5’ GGGATTGGGACCTCTTCTTAC

Real-time quantitative PCR (RT-qPCR)

72 hours after siRNA transfection, total RNA from the cells was extracted with the Trizol reagent (15596-028, Invitrogen) according to the manufacturer's protocol. The cDNA was synthesized using SuperScript II reverse transcriptase (180604014, Invitrogen) with 1 μg of total RNA in a 20 μL reaction. The resulting cDNA was diluted 1:20 in nuclease-free water, and 1 μL was used per qPCR reaction in triplicates. qPCR was carried out using Power SYBR Green PCR Master Mix (4367659, Thermo Fisher Scientific) on a CFX384 Real-Time PCR Detection System (Bio-Rad) including a non-template negative control. Amplification of *GAPDH* or *18S rRNA* was used to normalize the level of mRNA expression. The sequences of the primer pairs were listed in Table 3.3.1.

siRNA transfection and gene expression analysis

FOXM1-specific siRNAs and scrambled negative control siRNAs were synthesized by Integrated DNA Technologies (IDT, Coralville, IA, USA). 3.5 x 10^5 cells/well were seeded in 6-well plates and incubated at 37 °C overnight. The next day, 20 nM of each siRNA was transfected into the cells with Oligofectamine Transfection Reagent (12252011, Invitrogen) according to manufacturer's instructions. Culture media was added 6-8 hours after transfection without washing cells. 48 hours after siRNA transfection, cells were trypsinized and seeded in 6-well plates and kept overnight. Approximately 72 hours after siRNA transfection, cells were treated with vehicle, 1 μM or 5 μM (+)-JQ1 for 24 hours before the Western blot analysis. To check the downregulation of *FOXM1* expression, the transfected cells were collected to extract total RNA for qRT-PCR or proteins for the Western blot analysis at 72 hours after transfection. The sequences of siRNAs used are listed below:

FOXM1 siRNA#1: sense rGrUrGrCrCrArArCrCrGrCrUrArCrUrUrGrArCrArUrUrGGA, antisense rUrCrCrArArUrGrUrCrArArGrUrArGrCrGrGrUrUrGrGrCrArCrUrG; *FOXM1* siRNA#2: sense rGrCrGrCrUrArUrUrArGrArUrGrUrUrUrCrUrCrUrGrArUAA, antisense rUrUrArUrCrArGrArGrArArArCrArUrCrUrArArUrArGrCrGrCrArC. *c-MYC* siRNA#1: sense rArUrCrArUrUrGrArGrCrCrArArArUrCrUrUrArArArArAAA, antisense rUrUrUrUrUrUrUrArArGrArUrUrUrGrGrCrUrCrArArUrGrArUrArU; *c-MYC* siRNA#2: sense rCrGrArCrGrArGrArCrCrUrUrCrArUrCrArArArArArCrATC, antisense rGrArUrGrUrUrUrUrUrGrArUrGrArArGrGrUrCrUrCrGrUrCrGrUrC.

Cytotoxicity assay using Sulforhodamine B (SRB) and drug synergy studies

SRB assays were performed as previously described (Vichai and Kirtikara 2006) (Bastola, Neums et al. 2016) with some modifications shown below. For OVCA420*, OV90, and ES-2 cells, 3000 cells/well were seeded in 96-well plates and treated with drugs after12 hours of seeding. The cells were incubated for another 3 days. Dose-response curves and IC_{50} for each drug were determined by GraphPad Prism (ver. 6) with four parameters. All curves were constrained with 100% at the top. Synergy was determined by calculating the combination index (CI) obtained from the plate reading. CI was calculated based on dividing the expected additive effect by the observed effect.

Colony formation assay

For OVCA420*, OV90, and ES-2, 1000 cells were seeded in 6-well plates. For MDA-MB-436 rucaparib resistant cells (RR-1 and RR-2), 2000 or 3000 cells per well were seeded in 6-well plates. The cells were treated with drugs after 12 hours of seeding and further incubated for another 3 days before changing to fresh media. The medium was changed every 3 days to allow colonies to form. At the end of experiments, colonies were stained with SRB and imaged with Molecular

Imager ChemiDoc MP System (Bio-Rad). Stained SRB was later dissolved and measured with a plate reader. The analysis of clonogenic assay was performed in GraphPad Prism (ver. 6).

Statistical analysis

All data were analyzed using GraphPad Prism (ver. 6). Results were expressed as means ± standard error of the mean (SEM.) unless otherwise indicated. Differences between treatment regimens were analyzed by one-way ANOVA or two-tailed Student's t-test. $p \leq 0.05$ was considered to be statistically significant, *p=0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3.4 Results

3.4.1 FOXM1 knockdown results in the upregulation of c-MYC in ovarian cancer cells

FOXM1 has been shown to regulate DNA repair genes (Raychaudhuri and Park 2011). Therefore, we tested the effects of FOXM1 knockdown by siRNAs on the expression of known FOXM1 target genes in DNA repair pathway, including *BRCA1, BRCA2, BRIP1,* and *FANCF*. In ovarian cancer ES-2 cells at 72 hours after siRNA transfection, two independent *FOXM1* siRNAs caused more than 50% reduction of *FOXM1* mRNA levels (Figure 3.4.1.A) and a more dramatic decrease in protein levels (Figure 3.4.1.B). However, we did not observe a significant decrease in mRNA levels of *BRCA1*, *BRCA2,* and *FANCF* after *FOXM1* knockdown in these cells. This finding suggests that FOXM1 might not be the main transcription factor that regulates the expression of these genes, which is consistent with previous reports (Kwok, Peck et al. 2010). Meanwhile, we observed a slight downregulation of *BRIP1* mRNAs with both *FOXM1* siRNAs, which indicates the direct regulation of *BRIP1* by FOXM1 and consistent with a previous report (Monteiro, Khongkow et al. 2013). The slight downregulation also suggests that FOXM1 might be only one of many transcription factors regulating the expression of *BRIP1*. Taken together, we conclude

that FOXM1 downregulation is not sufficient to downregulate the tested target genes in the DNA repair pathway. Interestingly, we observed an increase in the levels of c-MYC protein after FOXM1 knockdown for 72 hours by siRNAs in ES-2 cell. The increase of c-MYC protein expression was suppressed with *MYC* siRNA transfection (Figure 3.4.1.B). In another cancer cell line OVCA420*, we also observed the upregulation of c-MYC at 72 hours after FOXM1 knockdown (Figure 3.4.1.C). These data suggest that FOXM1 knockdown results in an increase in c-MYC expression, which might potentially serve as a compensatory mechanism to regulate the expression of target genes regulated by both FOXM1 and c-MYC.

Figure 3.4.1

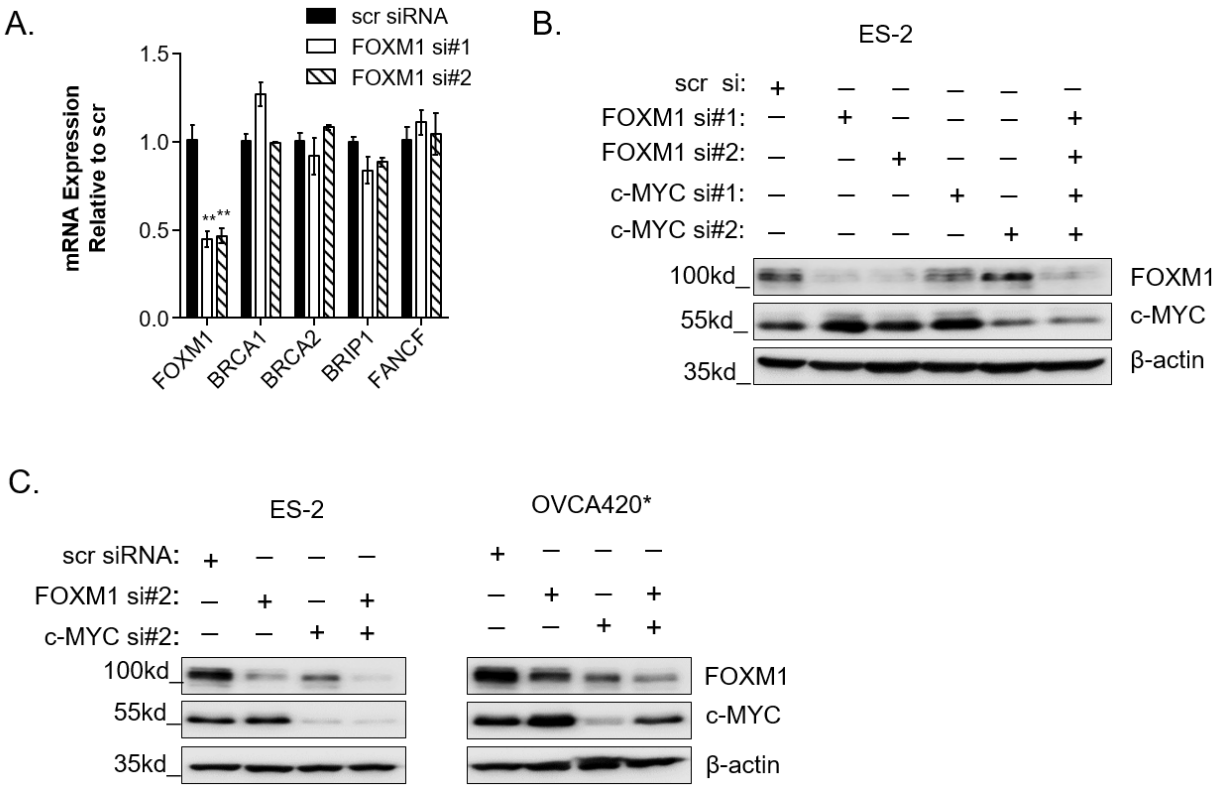


Figure 3.4.1: FOXM1 knockdown results in the upregulation of c-MYC in ovarian cancer cells. (A) FOXM1 knockdown is not sufficient to downregulate its target genes in HR pathway. ES-2 cells were transfected with scrambled siRNA (scr si) or two different *FOXM1* siRNAs for 72 hours. Total RNAs were extracted and reverse transcribed to generate cDNA for qRT-PCR. Data are shown as mRNA expression level relative to scr siRNA with mean ± SEM. Results shown as one representative experiment with triplicates. **(B-C)** *FOXM1* knockdown results in an increase in c-MYC expression. (B) 72 hours after siRNA transfection, ES-2 cells were collected for the Western blot analysis. Specific antibodies for FOXM1 and c-MYC were used, β-actin was used as a loading control. (C) Representative Western blots for ES-2 and OVCA420* cells. Experiments were done 72 hours after siRNA transfection. Results are representatives from at least three independent experiments.

3.4.2 DNA repair genes are regulated by both FOXM1 and c-MYC

Next, we are interested to know whether the target DNA repair genes are regulated by both transcription factors, FOXM1 and c-MYC. We analyzed data from the ENCODE (Encyclopedia of DNA Element) project available through the UCSC genome browser. We found 21,667 binding sites across the genome for FOXM1, and 96,959 binding sites for MYC. We observed 6,453 regions that have at least 70% overlap of binding sites for both FOXM1 and MYC. Next, we limited our analysis to the overlapping binding sites located in regulatory regions next to the target genes (10 kb upstream and 1 kb downstream from transcription start site), and we observed 62% of those overlapping binding sites are proximity to genes (Figure 3.4.2.A). Annotation of overlapping regions to proximal genes identified several DNA repair genes (Table 3.4.1), including *ATM*, *ATR*, *BRIP1*, *RAD51B*, *RAD51D*, *RAD50*, *XPC*, *XRCC3*, and others. The disease ontology analysis of proximal genes associated with the overlapping binding sites for FOXM1 and MYC suggests that one of the most significantly disease is hereditary breast and ovarian cancer (HBOC) (Figure 3.4.2.B). These results suggest that FOXM1 and MYC share target genes that are associated with hereditary breast and ovarian cancer and these target genes are involved in DNA repair, DNA damage response, HR pathway, and Fanconi anemia pathway. This finding provides us a rationale to target both FOXM1 and c-MYC to induce *"BRCAness"* in the PARP inhibitor resistant ovarian cancer cells and enhance effects of PARP inhibitor.

Figure 3.4.2

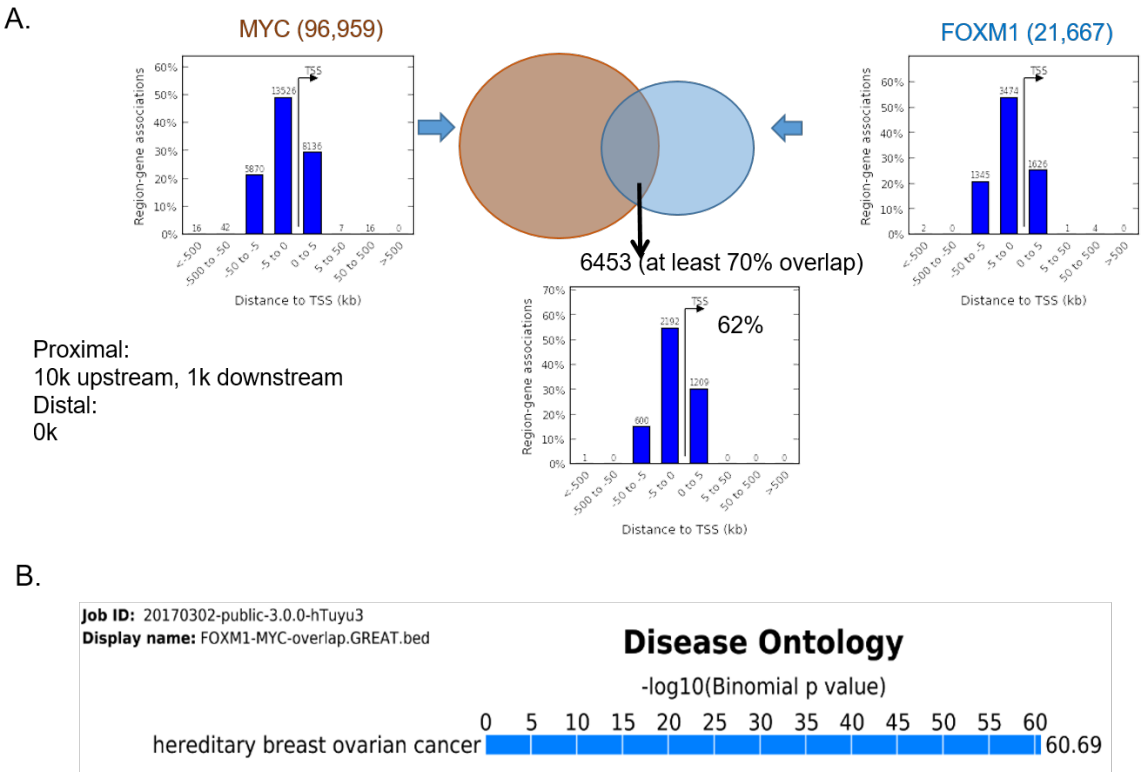


Figure 3.4.2: ENCODE data analysis reveals that DNA repair genes are regulated by both FOXM1 and c-MYC. (A) ENCODE data analysis indicates FOXM1 and MYC share 6,453 binding sites with at least 70% overlap of regions for both transcription factors. 62% of these sites are located proximal (10 kb upstream or 1 kb downstream) to the genes. **(B)** Disease ontology analysis of genes with overlapping regulatory regions for FOXM1 and MYC reveals that one of the significantly associated diseases is hereditary breast and ovarian cancer. The binomial p value is shown as –log10.

Table 3.4.1

Table 3.4.1 Genes associated with Disease Ontology for Hereditary Breast and Ovarian Cancer

Table: Genes with overlapped binding sites for MYC and FOXM1									
ATM	(+196)	**CSTF1**	(+9)	HRAS	(-1795)	**PALB2**	(-76)	**SSBP1**	(-375)
ATR	(-18)	CYP1B1	(-883)	JUNB	(-8718)	PIK3CA	(-618)	STAT1	(-6201), (-54)
AURKA	(-108)	ECT2	(+13)	**KAT5**	(-113)	**PLK1**	(-17)	TGFB1	(-254)
BABAM1	(-6341)	EPHB2	(-6301)	CCAR2	(+2)	**PTEN**	(-668), (+451)	**TOP2A**	(-80)
BLM	(+45)	**ERCC1**	(-4225), (-388)	KIF4A	(-42)	PTK2	(-1194)	**TOPBP1**	(-60)
BRIP1	(-36)	**ERCC5**	(+984)	**MDC1**	(-4960), (+268)	**RAD50**	(+76)	TSG101	(+276)
EMSY	(-2320)	ESR2	(-676)	**MLH1**	(+241)	**RAD51B**	(-2999)	UBE2I	(+382)
CASP10	(+160)	EZH2	(-322)	MYC	(-1974), (+198)	**RAD51D**	(-78)	UBE2L6	(-541)
CASP8	(+408)	**FAM175A**	(+509)	**NBN**	(+380)	**RAD52**	(+4)	**UBE2T**	(-96)
CCNT1	(+209)	**FANCD2**	(+4)	NCL	(+309)	RELA	(-150)	UBXN1	(+11)
CDC27	(-29)	FOXC1	(-5911)	NCOA3	(-103)	**RFC1**	(+20)	**XPC**	(-34)
CDK2	(+79)	GTF2I	(-270)	NME1	(-45)	RNASEL	(-6598)	XPO1	(-131)
CDKN1A	(+70)	**H2AFX**	(-6458), (-189)	NPM1	(+885)	**SMC1A**	(+274)	**XRCC3**	(-3393)
CHEK1	(-511)	HDAC1	(-191)	NR1H3	(-9138)	SP1	(-8843)	ZNF350	(-3)
COL1A1	(+928)	HMMR	(-110)	NUMB	(-307)				
# GREAT version 3.0.0 (Species assembly: hg19). Association rule: Basal+extension: 10000 bp upstream, 1000 bp downstream, 0 bp max extension, curated regulatory domains included.									

DNA repair genes are highlighted in bold. Values in parenthesis are distance in nucleotide base for overlapping binding sites for FOXM1 and MYC relative to transcription start site (TSS). Negative values represent upstream from TSS. Positive values represent downstream from TSS.

3.4.3 *FOXM1, MYC,* and *BRD4* are highly expressed in ovarian cancer and their expressions are related to each other

To corroborate our *in vitro* observation, we analyzed datasets from the Cancer Genome Atlas (TCGA). *FOXM1* and *MYC* are found to have higher median mRNA expression levels across 24 different types of cancer compared to normal tissue in TCGA datasets. Ovarian carcinomas have the second highest *FOXM1* expression among these 24 types of cancer (Figure 3.4.3.A). Similarly, ovarian carcinomas have the fourth highest *MYC* expression following after Uveal melanoma, colorectal, and head & neck cancer (Figure 3.4.3.B). Bromodomain-containing protein 4 (BRD4) is a member of the BET (bromodomain and extra-terminal domain) protein family and has been implicated in promoting gene transcription through interaction with the transcription elongation factor P-TEFb and RNA polymerase II (Itzen, Greifenberg et al. 2014). *MYC* expression is positively regulated by BRD4 (Delmore, Issa et al. 2011). Studies have shown that BRD4 also regulates *FOXM1* expression (Zhang, Ma et al. 2016). We also analyzed the expression of *BRD4* from TCGA datasets, and we found that *BRD4* also has a higher median expression in multiple types of cancer, with ovarian carcinomas having the highest expression (Figure 3.4.3.C), which is consistent with the observation from Zhang *et al* (2016) (Zhang, Ma et al. 2016). Meanwhile, the expression correlation analysis indicates that expression of *FOXM1* and *MYC* shows a mild negative correlation (Figure 3.4.3.D), which is consistent with our observation that *FOXM1* knockdown leads to upregulation of c-MYC (Figure 3.4.1.A-C). We also observed mild positive correlations between *BRD4* and *FOXM1* as well as between *BRD4* and *MYC*, which is consistent with previous reports (Delmore, Issa et al. 2011, Zhang, Ma et al. 2016). Taken together, these data suggest that *FOXM1, MYC,* and *BRD4* are highly expressed in ovarian cancer and BRD4 expression is positively correlated with FOXM1 and MYC, suggesting that targeting BRD4 may

suppress the expression of both FOXM1 and MYC and downregulate their downstream target genes involved in DNA repair. The downregulation of consequential downstream target genes in DNA repair pathway may induce a *"BRCAness"* like phenotype and enhance sensitivity to PARP inhibitors.

Figure 3.4.3

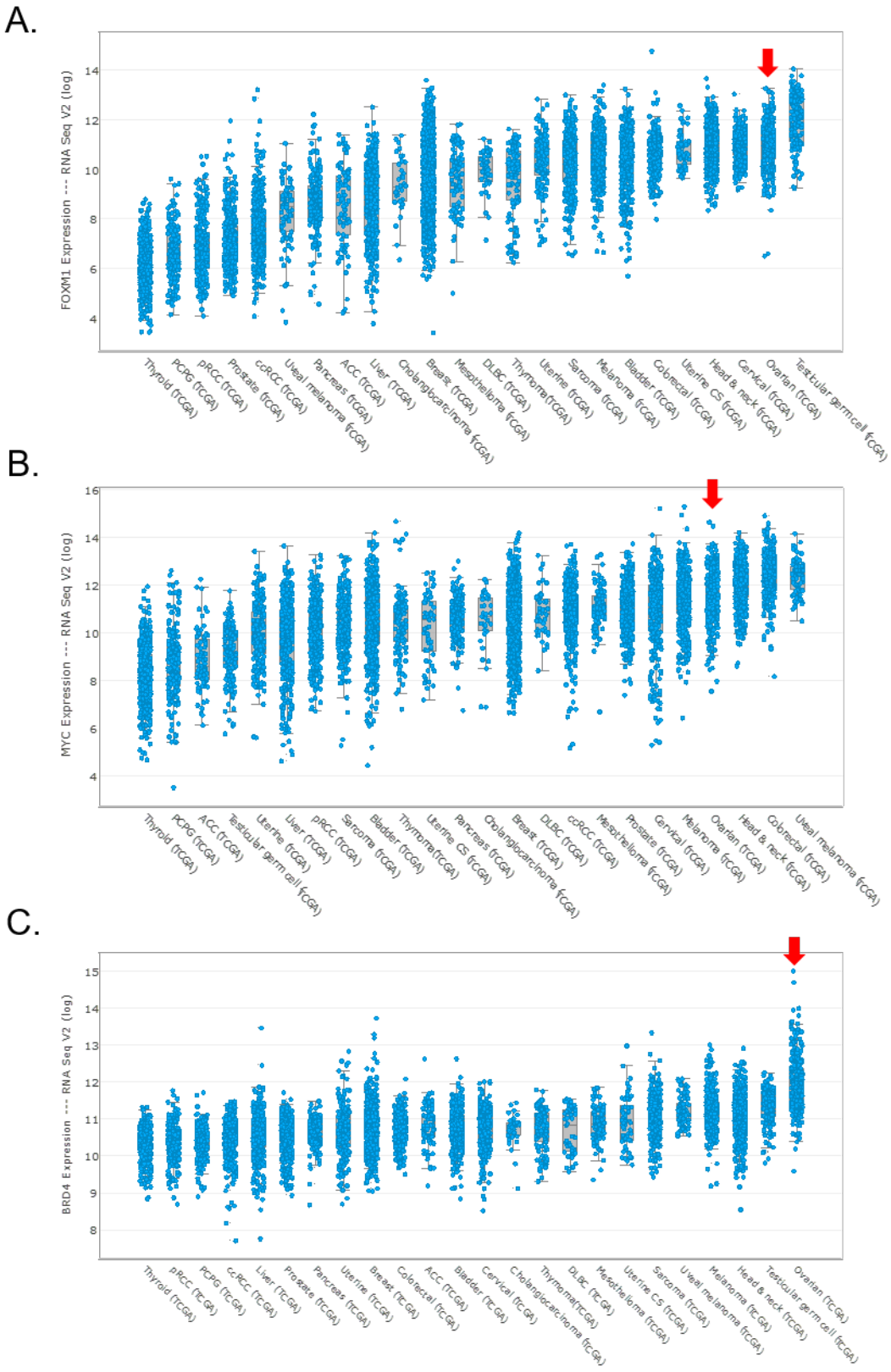
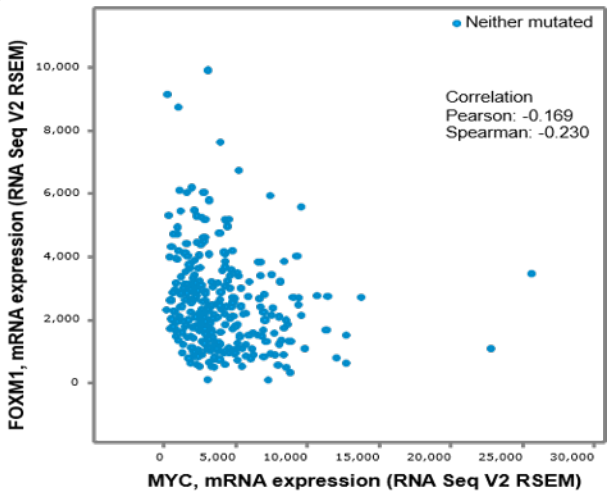
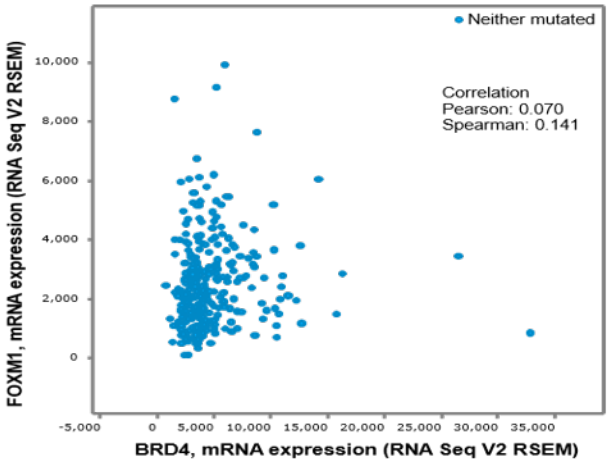

A.
FOXM1 Expression --- RNA Seq V2 (log)
14
12
10
8
6
4
Thyroid (TCGA)
PCPG (TCGA)
pRCC (TCGA)
Prostate (TCGA)
ccRCC (TCGA)
Uveal melanoma (TCGA)
Pancreas (TCGA)
ACC (TCGA)
Liver (TCGA)
Cholangiocarcinoma (TCGA)
Breast (TCGA)
Mesothelioma (TCGA)
DLBC (TCGA)
Thymoma (TCGA)
Uterine (TCGA)
Sarcoma (TCGA)
Melanoma (TCGA)
Bladder (TCGA)
Colorectal (TCGA)
Uterine CS (TCGA)
Head & neck (TCGA)
Cervical (TCGA)
Ovarian (TCGA)
Testicular germcell (TCGA)
B.
MYC Expression --- RNA Seq V2 (log)
16
14
12
10
8
6
4
Thyroid (TCGA)
PCPG (TCGA)
ACC (TCGA)
Testicular germ cell (TCGA)
Uterine (TCGA)
Liver (TCGA)
pRCC (TCGA)
Sarcoma (TCGA)
Bladder (TCGA)
Thymoma (TCGA)
Uterine CS (TCGA)
Pancreas (TCGA)
Cholangiocarcinoma (TCGA)
Breast (TCGA)
DLBC (TCGA)
ccRCC (TCGA)
Mesothelioma (TCGA)
Prostate (TCGA)
Cervical (TCGA)
Melanoma (TCGA)
Ovarian (TCGA)
Head & neck (TCGA)
Colorectal (TCGA)
Uveal melanoma (TCGA)
C.
BRD4 Expression --- RNA Seq V2 (log)
15
14
13
12
11
10
9
8
Thyroid (TCGA)
pRCC (TCGA)
PCPG (TCGA)
ccRCC (TCGA)
Liver (TCGA)
Prostate (TCGA)
Pancreas (TCGA)
Uterine (TCGA)
Breast (TCGA)
Colorectal (TCGA)
ACC (TCGA)
Bladder (TCGA)
Cervical (TCGA)
Cholangiocarcinoma (TCGA)
Thymoma (TCGA)
DLBC (TCGA)
Mesothelioma (TCGA)
Uterine CS (TCGA)
Sarcoma (TCGA)
Uveal melanoma (TCGA)
Melanoma (TCGA)
Head & neck (TCGA)
Testicular germ cell (TCGA)
Ovarian (TCGA)

Figure 3.4.3 Cont.

D.



E.



F.

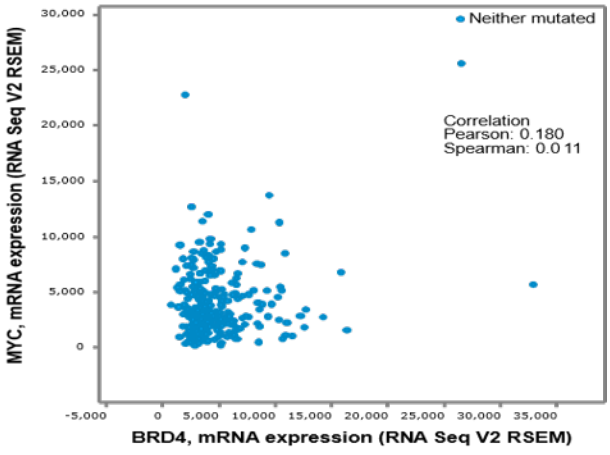


Figure 3.4.3: *FOXM1, MYC,* and *BRD4* are highly expressed in ovarian cancer. (A-C) The analysis of TCGA datasets reveals high median expression of mRNAs of *FOXM1* (A), *MYC* (B), and *BRD4* (C) in many types of tumors, including ovarian tumors, compared to normal tissue. **(D-F)** Correlation analysis reveals that expression of *FOXM1* and *MYC* shows a mild negative correlation, whereas *FOXM1* and *MYC* expression are mildly positive-correlated with *BRD4* expression.

3.4.4 BET inhibitor (+)-JQ1 induces *"BRCAness"* in ovarian cancer cells

BET inhibitors are small molecules that bind to the BET bromodomains and block the interaction of BET proteins (BRD2,3,4 and BRDT) with acetylated lysine residues on histone tails to regulate gene transcription (Garnier JM, et al., 2014; Junwei S, et al., 2014). They have been shown to block c-MYC expression in pre-clinical cancer models. BET inhibitors have also been reported to disrupt FOXM1 pathway and show efficacy in ovarian carcinomas (Zhang et al., 2016). We propose to use BET inhibitor to inhibit BRD4 to achieve downregulation of both c-MYC and FOXM1, thereby suppressing the expression of their downstream target DNA repair genes, especially the HR genes, such as *BRCA1* and *RAD51*, and inducing *"BRCAness"* to sensitize cancer cells to PARP inhibitor.

We used one of the pre-clinical BET inhibitors, (+)-JQ1, which has been shown to work in nanomolar concentrations (Delmore, Issa et al. 2011). First, we tested whether (+)-JQ1 can downregulate c-MYC expression induced by *FOXM1* knockdown. In ES-2 cell, we observed downregulation of both FOXM1 and c-MYC with (+)-JQ1 treatment in the control siRNA-transfected group, with FOXM1 being downregulated to a greater extent than c-MYC (Figure 3.4.4.A). (+)-JQ1 treatment also attenuated the c-MYC expression induced by the *FOXM1* knockdown at 72 hours, and (+)-JQ1 further decreases FOXM1 expression (Figure 3.4.4.A). The extent of c-MYC downregulation is not as robust as FOXM1, suggesting that c-MYC expression is not primarily dependent on BET proteins in these cells. Next, we tested if (+)-JQ1 can downregulate HR genes, *BRCA1* and *RAD51*, in three cancer cell lines, OVCA420*, OV90, and ES-2, that are relatively insensitive to olaparib (as shown in chapter 2). Twenty-four hours after (+)-JQ1 treatment, we saw a consistent dose-dependent downregulation of FOXM1 in all three cell lines (Figure 3.4.4.B), and a consistent efficient downregulation of c-MYC with higher

concentrations of (+)-JQ1 in OVCA420* and OV90 cells (Figure 3.4.4.B). At the same time, we examined the expression of *BRCA1* and *RAD51*, whose expression is regulated by both FOXM1 and c-MYC according to our analysis of ENCODE data (Figure 3.4.4.C). We saw a consistent dose-dependent downregulation of both BRCA1 and RAD51 by (+)-JQ1 in all three cell lines (Figure 3.4.4.B). We also performed ChIP (chromatin immunoprecipitation) analysis with FOXM1 or c-MYC pull down after (+)-JQ1 treatment in OVCA420* cells and observed that the binding of both FOXM1 and c-MYC to the promoters of both *BRCA1* and *RAD51* decreased significantly in cells treated with 5 μM (+)-JQ1 for 24 hours compared to vehicle-treated groups (Figure 3.4.4.D). These data suggest that the downregulation of *BRCA1* and *RAD51* by (+)-JQ1 is in part due to a decrease in transactivation by both FOXM1 and c-MYC through decreased binding to their promoter regions.

Figure 3.4.4

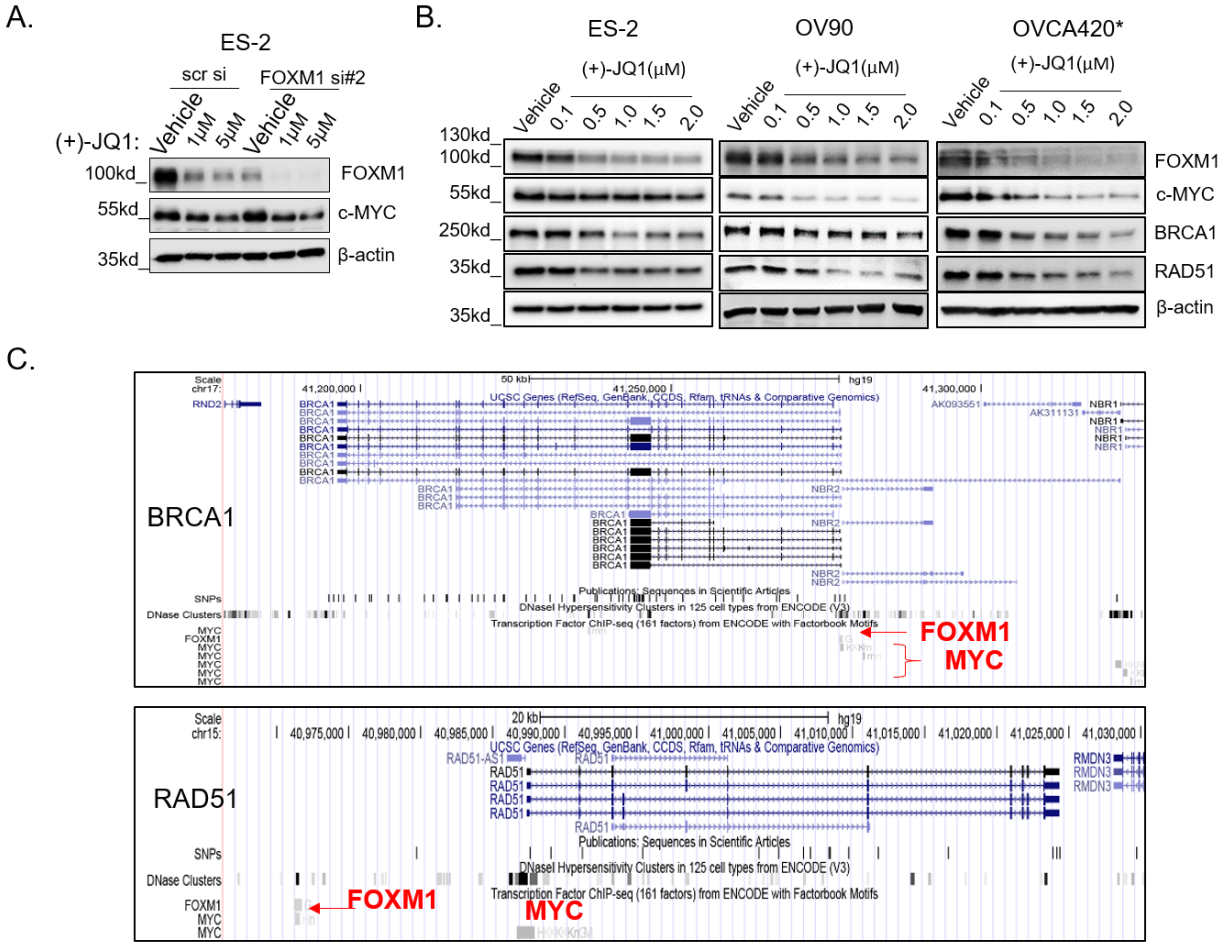

A.
ES-2
scr si
FOXM1 si#2
(+)-JQ1:
Vehicle
1μM
5μM
Vehicle
1μM
5μM
100kd
55kd
35kd
FOXM1
c-MYC
β-actin
B.
ES-2
OV90
OVCA420*
(+)-JQ1(μM)
Vehicle
0.1
0.5
1.0
1.5
2.0
130kd
100kd
55kd
250kd
35kd
35kd
FOXM1
c-MYC
BRCA1
RAD51
β-actin
C.
BRCA1
RAD51
FOXM1
MYC
D.
FOXM1_ChIP
c-MYC_ChIP
Vehicle
JQ1 5 μM
% of Enrichment Relative to Input
CCNB1
BRCA1
RAD51

Figure 3.4.4: BET inhibitor, (+)-JQ1, induces *"BRCAness"* in ovarian cancer cells. (A) (+)-JQ1 attenuates c-MYC expression induced by the FOXM1 knockdown. Forty-eight hours after siRNA transfection, vehicle, 1 μM or 5 μM of (+)-JQ1 were added to ES-2 cells and treated for another 24 hours before the Western blot analysis. **(B)** (+)-JQ1 downregulates FOXM1, c-MYC and their targets, *BRCA1* and *RAD51*, in a dose-dependent manner in three different cell lines, ES-2 (B), OV90 (C) and OVCA420* (D). **(C)** Both FOXM1 and c-MYC have binding sites in promoter regions of *BRCA1* and *RAD51*. The image file was generated from ENCODE data in UCSC genome browser. **(D)** ChIP-qPCR analysis indicates the decreased binding of both FOXM1 and c-MYC to promoter sequences of *BRCA1* and *RAD51* in OVCA420* cells that were treated with (+)-JQ1. Cells were treated with vehicle or 1 μM (+)-JQ1 for 24 hours before ChIP analysis. *CCNB1* is a positive control for FOXM1 binding. Data are shown as % of enrichment relative to input with mean ± SEM. Statistics analysis was done using the Student's t-test. **p<0.01, ****p<0.0001. Results are representatives from three independent experiments.

3.4.5 (+)-JQ1 sensitizes cancer cells with primary resistance to PARP inhibitor

Next, we tested whether (+)-JQ1 can make PARP inhibitor resistant cancer cells more sensitive. First, we performed a short-term cell viability assay to test the effects of (+)-JQ1 in three different cancer cells, and we observed a dose-dependent decrease in cell viability with (+)-JQ1 treatment (Figure 3.4.5.A). We also observed that the IC_{50} for (+)-JQ1 in these cells are in micromolar range, and ES-2 cell is more sensitive to (+)-JQ1 compared to the other two cells. Next, we used SRB assay to test the effects of (+)-JQ1 in combination with the FDA-approved PARP inhibitor, olaparib. We observed a weak synergy between (+)-JQ1 and olaparib in ES-2 and OV90 cells, and an additive effect in OVCA420* cells (Figure 3.4.5.B). We also performed a long-term colony formation assay (Figure 3.4.5.C-E). (+)-JQ1 treatment by itself leads to a reduction of colony formation in all three cells and resulted in a complete inhibition of colony formation when combined with sub-lethal doses of olaparib. We calculated the combination indexes and observed a moderate synergistic effect between (+)-JQ1 and olaparib. These data suggest that (+)-JQ1 enhanced the cytotoxic effect of olaparib in these cancer cells with primary resistance to PARP inhibitor.

Figure 3.4.5

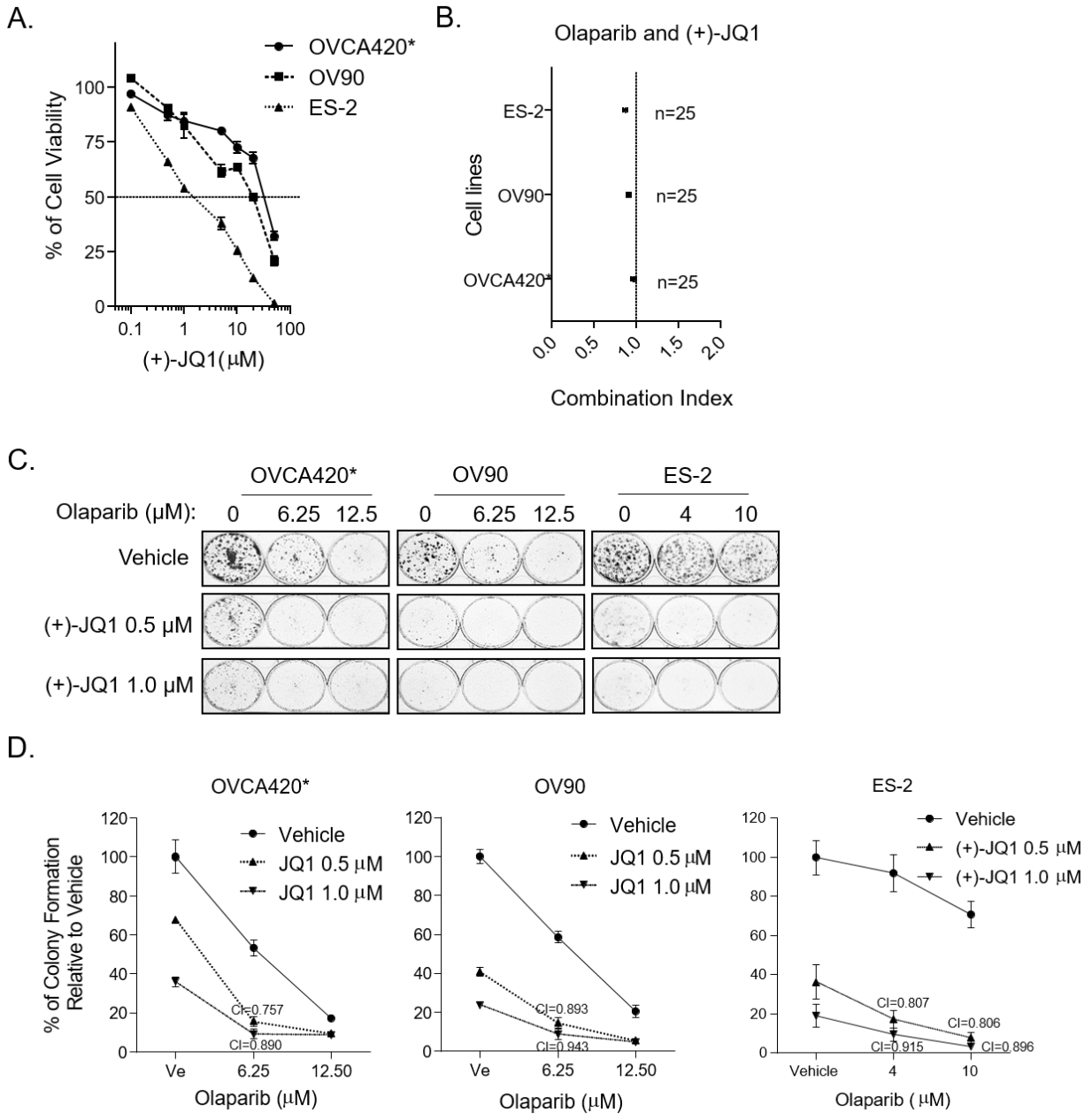


Figure 3.4.5: (+)-JQ1 sensitizes PARP inhibitor-insensitive cells to olaparib. A) (+)-JQ1 dose-dependently decreases cell viability in all three cancer cells. Cells were treated with increasing concentrations of (+)-JQ1 for 3 days in 96-well plates before Sulforhodamine B (SRB) cell viability assay. Data are shown as % of cell viability relative to the vehicle with mean ± SEM. Results represent one

representative experiments with triplicates. **B)** (+)-JQ1 and olaparib showed additive or weak synergistic effects when combined together in cancer cells by short-term SRB assay. Results represent one representative experiments with four replicates. **C-D)** Long-term colony formation assay showed moderate synergistic effects of (+)-JQ1 and olaparib. OVCA420*, OV90, and ES-2 cells were seeded in 6-well plates and treated with different concentrations of the single drug, olaparib or (+)-JQ1, or different combinations of two drugs. Drugs were washed away 3 days later, and cells were fed with fresh media every 3-4 days for two weeks. Colonies were stained with SRB, imaged and counted. CI was calculated and indicated in the line graph for each combination. Line graph data are shown as % of colony formation relative to the vehicle with mean ± SEM. Results were from one representative experiment with duplicates.

3.4.6 (+)-JQ1 enhances effects of PARP inhibitor in cancer cells with acquired resistance to rucaparib

To test whether BET inhibitor can resensitize cancer cells with acquired resistance to PARP inhibitor, we used rucaparib-resistant cancer cells that were derived from a continuous exposure to PARP inhibitor rucaparib (Johnson, Johnson et al. 2013), named as RR-1 and RR-2. Using Western blot, we observed that (+)-JQ1 treatment consistently lead to decreased FOXM1 expression in two different resistant clones, however, we only saw c-MYC being downregulated moderately with a high concentration of (+)-JQ1 in RR-1 and no decrease in RR-2 (Figure 3.4.6.A). RAD51 protein was consistently downregulated by (+)-JQ1 treatment. The resistance to rucaparib was mediated by the stabilization of the mutant BRCA1 (mtBRCA1) protein in these cells, and the resistance can be overcome by HSP90 inhibitor, which leads to the downregulation of stabilized mtBRCA1 (Johnson, Johnson et al. 2013). Interestingly, we also saw a dramatic decrease of mtBRCA1 protein level with (+)-JQ1 treatment (Figure 3.4.6.A). Next, we performed colony formation assay to assess the effect of combining (+)-JQ1 and rucaparib. Similar to PARPi-insensitive cancer cells, we observed a dose-dependent decrease of colony formation in these resistant cells with (+)-JQ1 treatment, with RR-1 being more responsive than RR-2. This is consistent with the Western blot results that demonstrated c-MYC is not downregulated by (+)-JQ1 treatment in RR-2 but moderately downregulated in RR-1 (Figure 3.4.6.A). Consistent with the downregulation of both FOXM1 and c-MYC by (+)-JQ1 treatment, RR-1 showed a greater extent of the decrease in colony formation (Figure 3.4.6.B-C). Strikingly, in combinations of 1 μM rucaparib with 500 nM (+)-JQ1, or 2.5 μM rucaparib with 300 nM (+)-JQ1, the colony formation was completely inhibited in RR-1. In RR-2, combinations of 1 μM (+)-JQ1 and sub-lethal doses of rucaparib also completely inhibited colony formation (Figure 3.4.6.D-E). Combination indexes

indicate an overall moderate synergistic effect between (+)-JQ1 and rucaparib (Figure 3.4.6.C and Figure 3.4.6.E). Taken together, these data suggest that (+)-JQ1 treatment can help restore sensitivity to rucaparib in cancer cells with an acquired resistance to rucaparib in part by downregulating FOXM1 and c-MYC, *RAD51* and *mtBRCA1*. These results indicate the potential of using BET inhibitor to overcome PARP inhibitor resistance in cancer cells.

Figure 3.4.6

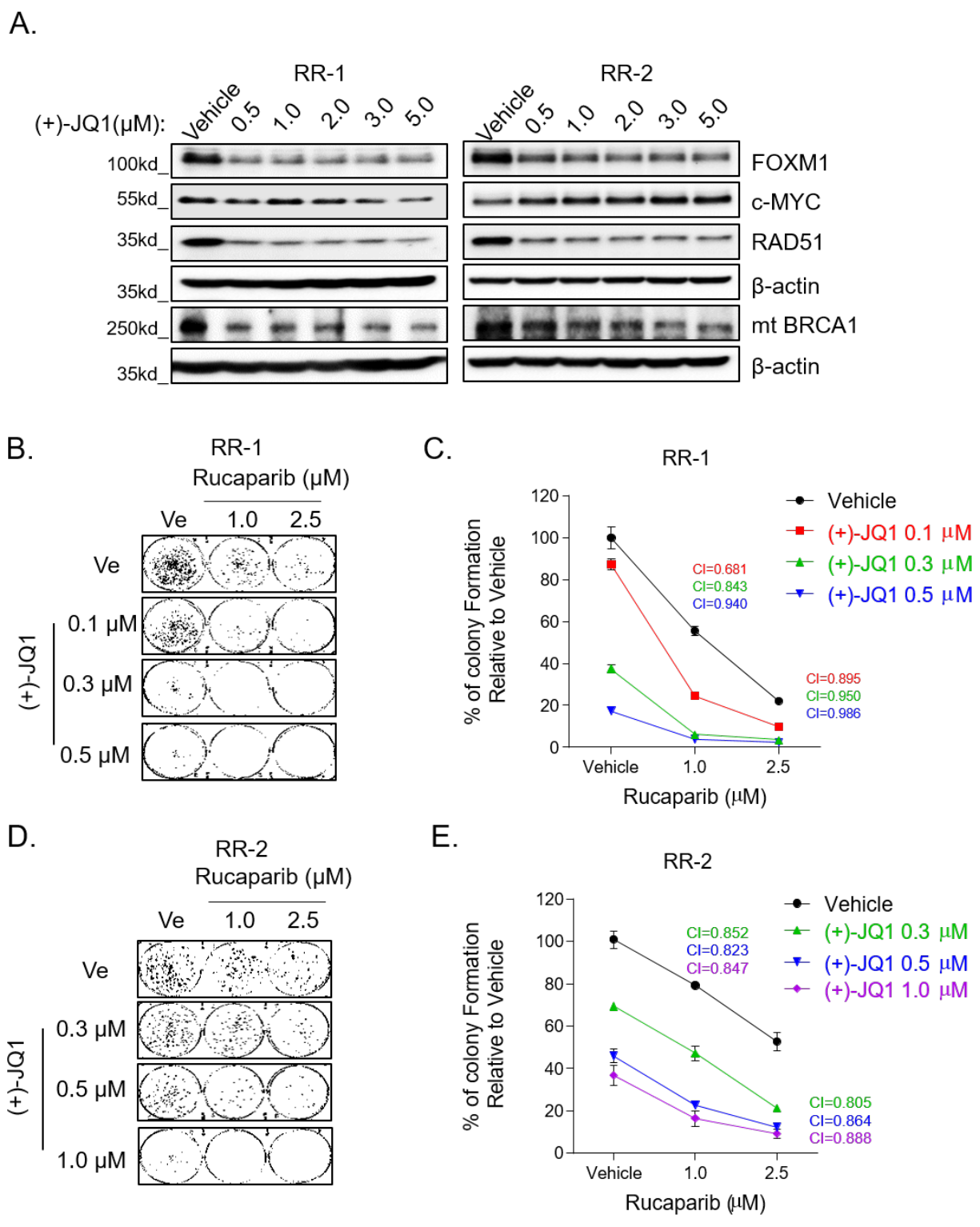

A.
RR-1
RR-2
(+)-JQ1(μM):
Vehicle
0.5
1.0
2.0
3.0
5.0
100kd_
55kd_
35kd_
35kd_
250kd_
35kd_
FOXM1
c-MYC
RAD51
β-actin
mt BRCA1
β-actin
B.
RR-1
Rucaparib (μM)
Ve
1.0
2.5
(+)-JQ1
0.1 μM
0.3 μM
0.5 μM
C.
RR-1
% of colony Formation Relative to Vehicle
Vehicle
(+)-JQ1 0.1 μM
(+)-JQ1 0.3 μM
(+)-JQ1 0.5 μM
CI=0.681
CI=0.843
CI=0.940
CI=0.895
CI=0.950
CI=0.986
Rucaparib (μM)
D.
RR-2
Rucaparib (μM)
(+)-JQ1
1.0 μM
E.
RR-2
Vehicle
(+)-JQ1 0.3 μM
(+)-JQ1 0.5 μM
(+)-JQ1 1.0 μM
CI=0.852
CI=0.823
CI=0.847
CI=0.805
CI=0.864
CI=0.888

Figure 3.4.6: (+)-JQ1 enhances the effects of PARP inhibitor in cancer cells with an acquired resistance to rucaparib. A) (+)-JQ1 treatment decreased the expression of FOXM1, c-MYC, RAD51 and mtBRCA1 in rucaparib-resistant cells. Cells were treated with (+)-JQ1 at indicated concentrations for 36 hours. Representative results were shown from three independent experiments. **B-E)** colony formation assay to test the combined effect of (+)-JQ1 and rucaparib. Representative images and quantification of colonies were shown. Data are shown as mean ± SEM. CI is also indicated in the graph. Results were shown from one representative experiment with duplicates.

3.4.7 PROTAC-based BET domain protein degrader downregulates *BRCA1* and *RAD51*

As a BET inhibitor, (+)-JQ1 has not been evaluated in the clinic because of its short half-life observed in preclinical *in vivo* study *(Filippakopoulos, Qi et al. 2010)*. Considering the potential clinical use of the BET inhibitor in combination with PARP inhibitor, we tested a recently developed PROTAC (proteolysis targeting chimera)-based BET domain protein degrader (ZBC260), which has been shown to degrade BRD proteins with a greater potency (Zhou, Hu et al. 2017). We tested the effects of ZBC260 in three different cell lines and observed that ZBC260 efficiently downregulates BRD4 starting from as low as 3 nM, and BRD4 was completely depleted with concentrations > 10 nM (Figure 3.4.7.A-C). These preliminary results suggest that ZBC260 is very efficient in suppressing and inhibiting BRD4 in our cells. In all three cell lines, FOXM1 was consistently downregulated by ZBC260 following a decrease of BRD4 (Figure 3.4.7.A-C). However, only in OVCA420* and OV90, we saw downregulation of c-MYC (Figure 3.4.7.A-B). Tested target genes of FOXM1 and c-MYC, such as *BRCA1* and *RAD51*, were also consistently downregulated following ZBC260 exposure to a greater extent at drug concentrations that are lower than (+)-JQ1 (Figure 3.4.4.B-D). We also tested the drug with short-term SRB assay and observed a dose-dependent reduction of cell viability with much lower concentrations of ZBC260 compared to (+)-JQ1 (Figure 3.4.5.A). Similar to (+)-JQ1, three cells responded to ZBC260 differently, with ES-2 cell being more sensitive than the other two cell lines (Figure 3.4.7.D). These data suggest that BET protein degrader is effective in downregulating FOXM1, c-MYC, and DNA repair genes, such as *BRCA1* and *RAD51* and is more potent than (+)-JQ1.

Figure 3.4.7

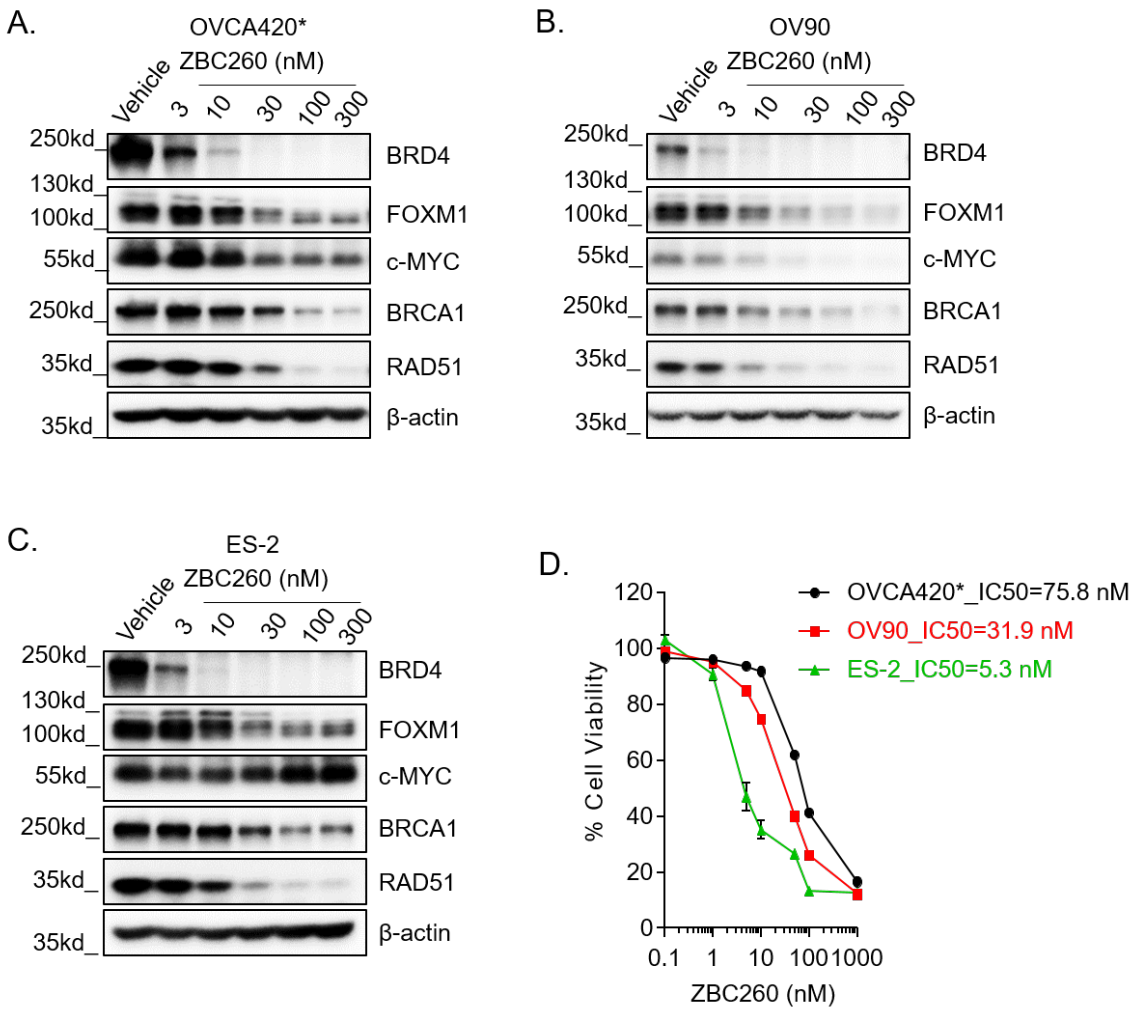


Figure 3.4.7: PROTAC BET domain protein degrader ZBC260 downregulates *BRCA1* and *RAD51*. **A-C)** Western blot shows the effect of ZBC260 in downregulating FOXM1, c-MYC, BRCA1, and RAD51. OVCA420* (A), OV90 (B) and ES-2 (C) were treated with vehicle, 3, 10, 30, 100 or 300 nM of ZBC260 for 24 hours. Results represent one experiment from three independent experiments. **(D)** SRB cell viability assay with ZBC260. A dose-dependent decrease of cell viability with ZBC260 treatment is observed in all three cells. Data are shown as percent (%) of cell viability relative to the vehicle with mean ± SEM. IC_{50}s estimated from dose-response curves are shown. Results shown as one representative experiment with triplicates.

3.5 Discussion

In this chapter, we demonstrated that exposure of ovarian cell line to BET inhibitors can lead to suppression of the expression of FOXM1 and c-MYC and downregulate the expression of downstream overlapping target genes, *BRCA1* and *RAD51,* which are involved in the HR pathway. Furthermore, we showed that the BET inhibitor, (+)-JQ1 can enhance the effect of PARP inhibitors in cancer cells with either primary or acquired resistance to PARP inhibitors. These data suggest that (+)-JQ1, as a BET inhibitor induces *"BRCAness"* in HR-proficient cancer cells to overcome PARP inhibitor resistance. Furthermore, BET inhibitor may be combined with PARP inhibitor to potentiate the effect of PARP inhibitor in patients with HR-proficient tumors.

In chapter 2, I showed that FOXM1 expression contributes to PARP inhibitor resistance, and upregulation of FOXM1 positively regulates HR genes, including *BRCA1* and *RAD51,* to enhance DNA repair. We noted that PARP inhibitor resistance was reversed by FOXM1 inhibition with either siRNAs or FOXM1 inhibitor, thiostrepton. Thiostrepton treatment leads to downregulation of HR genes. In this chapter, using *FOXM1* specific siRNA, we saw consistent downregulation of FOXM1 expression; however, its target genes, such as *BRCA1, BRCA2,* and *FANCF,* remained unchanged. Previous reports also failed to see the downregulation of FOXM1 target genes, such as *BRCA2* and *XRCC3,* following the FOXM1 knockdown (Kwok, Peck et al. 2010), suggesting that other transcription factors also regulate the expression of these genes. Interestingly, following FOXM1 knockdown, we saw upregulation of c-MYC in two different cell lines. The analysis of ENCODE data showed that thousands of genes have binding sites for both FOXM1 and c-MYC in their regulatory regions. Furthermore, there are 6,453 shared binding sites of FOXM1 and c-MYC that show at least 70% sequence overlap, including a list of DNA repair genes, such as *ATM, ATR, BRIP1, RAD50,* and etc. Also, disease ontology analysis of these genes suggests that one of

the most significantly associated diseases is the hereditary breast and ovarian cancer. Based on these observations, we hypothesized that FOXM1 and c-MYC co-regulate the expression of HR genes, such as *BRCA1* and *RAD51*, and that inhibition of both genes is necessary to decrease the expression of HR genes, to induce *"BRCAness"*, and to sensitize resistant cancer cells to PARP inhibitors.

BET inhibitor (+)-JQ1 has been shown to successfully inhibit c-MYC expression and MYC pathway in preclinical studies (Delmore, Issa et al. 2011). Recent studies also indicate BET inhibitors, (+)-JQ1 and iBET, downregulate FOXM1, although the molecular mechanism is not well-characterized (Zhang, Ma et al. 2016). Therefore, we tested the effect of BET inhibitor in cancer cells. (+)-JQ1 is a widely used BET inhibitor for *in vitro* studies, and we demonstrated that it can attenuate c-MYC expression induced by the FOXM1 knockdown. We also observed a consistent downregulation of both FOXM1 and c-MYC, as well as the downregulation of *BRCA1* and *RAD51* in at least two different cancer cells. Interestingly, compared to the other two cell lines, c-MYC in ES-2 cell was not efficiently downregulated even with high concentrations of (+)-JQ1. This inefficient suppression of c-MYC expression by (+)-JQ1 suggests that c-MYC expression in this cell line might not be solely dependent on BET proteins. In contrast, FOXM1 expression was consistently downregulated by exposure to (+)-JQ1, suggesting that FOXM1 expression is dependent on BET proteins in these cells. We also tested the cytotoxic effect of (+)-JQ1 in breast cancer cells with the acquired resistance to PARP inhibitor rucaparib. The two resistant cells were derived from the originally sensitive MDA-MB-436 breast cancer cell with a chronic exposure to rucaparib (Johnson, Johnson et al. 2013). The main mechanism of resistance in these cells is the restoration of HR function by the stabilization of mutant BRCA1 (mtBRCA1) protein, which otherwise was quickly degraded in MDA-MB-436 cells. From the original study (Johnson,

Johnson et al. 2013), the resistance in these cells can be overcome by HSP90 inhibitor, which leads to HSP90 inhibition and destabilization of mtBRCA1, resulting in HR deficiency and restoration of PARP inhibitor sensitivity. Interestingly, we found that (+)-JQ1 treatment resulted in downregulation of the originally stabilized mtBRCA1 protein in these cells, and this effect might be a result of decreased transcription caused by inhibition of FOXM1 and c-MYC. However, further studies are needed to unveil how (+)-JQ1 treatment led to decreased mtBRCA1 in these resistant cells.

Results from the short-term cell viability assay indicate a weak synergistic or additive effect between (+)-JQ1 and olaparib or rucaparib in cancer cells with primary or acquired resistance to PARP inhibitors, respectively. However, the long-term colony formation assay showed stronger synergistic interactions between (+)-JQ1 and PARP inhibitors. Colony formation assay is more relevant to our current study because drug effects of PARP inhibitors rely on cell cycling (Stordal, Timms et al. 2013). Although the synergy we observed is not very strong, combinations of sub-lethal doses of (+)-JQ1 and PARP inhibitor markedly suppress colony formation, suggesting the effectiveness of BET inhibitor in reducing the development of PARP inhibitor resistance.

Even though (+)-JQ1 had shown effectiveness in both *in vitro* and *in vivo* studies, it's potential use for clinical evaluation was reconsidered because of its short half-life *in vivo* (Filippakopoulos, Qi et al. 2010). Based on our observations that the combination of (+)-JQ1 and PARP inhibitors give better therapeutic effects, it is important test the efficacy of this combination in clinical trials, and clinical trials rely on the availability of an effective and safe BET inhibitor candidate. As a result, we tested effects of a recently developed PROTAC BET protein degrader, ZBC260, which has been tested as a single agent for *in vivo* studies (Zhou, Hu et al. 2017), but not in combination with PARP inhibitor. Our data showed that it is effective in downregulating

FOXM1, c-MYC, BRCA1 and RAD51. It also inhibited cell growth in a dose-dependent manner with IC_{50}s in the nanomolar range, suggesting that it is more potent than (+)-JQ1. In the future, the combined effects of ZBC260 and PARP inhibitors should be tested in cells with the primary or acquired resistance to PARP inhibitors, followed by the determination of the combined effects in patient-derived xenograft models. PARP inhibitors in combination with (+)-JQ1 have already been tested in several tumor xenograft models in mouse (Karakashev, Zhu et al. 2017, Yang, Zhang et al. 2017) and patient-derived xenograft (Garcia, Miller et al. 2016). Additional studies should focus on determining the combined effect in patient-derive xenograft models derived from HR-proficient cancer to demonstrate the extent to which BET degraders can extend the clinical utility of PARP inhibitors in treating patients with HR-proficient cancers.

Overall, in this chapter, we showed that BET inhibitors can inhibit the FOXM1-MYC nexus in cancer cells, downregulate BRCA1 and RAD51 to induce *"BRCAness"*, and that (+)-JQ1 treatment can sensitize resistant cells to PARP inhibitors, suggesting that the combination of BET inhibitor and PARP inhibitor might be a new strategy to enhance therapeutic effects of PARP inhibitors.

Chapter 4. Genome-scale CRISPR knockout screen identifies TIGAR as a novel modifier of PARP inhibitor sensitivity

4.1 Abstract

Cancer cells harboring homologous recombination defects (HRD) are shown to be sensitive to poly (ADP-ribose) polymerase (PARP) inhibitors. However, to which extent the therapeutic potential of PARP inhibitor can be explored still remains an active area of research. One of the key challenges is fully understanding of the molecular mechanisms that lead to PARP inhibitor sensitivity. In this study, using the sensitivity of PARP inhibitor olaparib as a surrogate, we performed a CRISPR/Cas9 based genome-scale loss-of-function screen and identified *C12orf5*, a gene that encodes a metabolic regulator, *TP53* induced glycolysis and apoptosis regulator (TIGAR) as a novel potential genetic determinant of PARP inhibitor response. Further studies showed that several mechanisms contribute to olaparib sensitivity in *TIGAR* knockdown cells: (1) the increase in intracellular reactive oxygen species (ROS) level leads to enhanced DNA damage after olaparib treatment; and (2) induction of *"BRCAness"* by downregulation of *BRCA1* and the Fanconi anemia (FA) pathway. In addition, *TIGAR* knockdown inhibits cell growth by induction of senescence. Concomitantly, *TIGAR* knockdown results in the reduced efficacy in spheroid formation and the enhanced therapeutic effects of olaparib. Finally, TCGA data analysis showed that *TIGAR* is amplified in different types of cancer including ovarian cancer, and higher expression of *TIGAR* is associated with poor overall survival in high-grade serous ovarian cancer. Taken together, this study showed that TIGAR negatively regulates PARP inhibitor sensitivity and is a novel potential therapeutic target to explore as mono or combination therapies for treating cancers.

4.2 Introduction

Following the discovery that poly (ADP-ribose) polymerase (PARP) inhibitors cause synthetic lethality in cancer cells with *BRCA1* or *BRCA2* deficiencies (Bryant, Schultz et al. 2005, Farmer, McCabe et al. 2005), a number of PARP inhibitors were approved by the Federal Drug Administration as anti-cancer drugs for various cancers with either germline or somatic *BRCA1* and *BRCA2* mutations.. It is now well accepted that PARP inhibitors are also active in cancers with the so-called *"BRCAness"* phenotype (Turner, Tutt et al. 2004, Lord and Ashworth 2016). This phenotype is found in tumors with homologous recombination (HR) defects resulting from alterations of components other than *BRCA1* or *BRCA2* in the HR pathway (McCabe, Turner et al. 2006, Gaymes, Mohamedali et al. 2013, Gilardini Montani, Prodosmo et al. 2013, Bajrami, Frankum et al. 2014, Smith, Hampton et al. 2015). In addition, alterations in genes not directly related to the HR pathway, such as *PTEN* loss (Mendes-Pereira, Martin et al. 2009) and translocation in *TMPRSS2-ERG* (Brenner, Ateeq et al. 2011) and *EWSR1-FLI1* (Garnett, Edelman et al. 2012), also result in the increased sensitivity of PARP inhibitors. These findings suggest that other molecular pathways unrelated to the HR pathway may also contribute to PARP inhibitor sensitivity. A better understanding of genetic determinants that contribute to PARP inhibitor sensitivity will extend the clinical utility of PARP inhibitors.

One of the challenges to further development of PARP inhibitors is to fully understand the molecular mechanisms contributing to PARP inhibitor sensitivity (Yap, Sandhu et al. 2011). To address this challenge, several studies have reported candidate genes that cause synthetic lethality with PARP inhibitors from genome-wide RNAi profiling and functional studies (Lord, McDonald et al. 2008, Turner, Lord et al. 2008, Bajrami, Frankum et al. 2014). These studies identified genes involved in DNA damage response (DDR) and repair pathways such as *BRCA1*, *NBN*, *FANCD*,

FANCC, *RAD51*, *LIG3*, *RAD51C*, *RAD51D*, *RAD21*, *ESCO1*, and *SMC3*, as well as genes involved in replication and cell cycle progression such as MCM proteins, *TOP3A*, *POLB*, and *CDK7* as modulators of sensitivity to PARP inhibitors (Bajrami, Frankum et al. 2014). However, the clinical benefit of targeting these genes to enhance PARP inhibitor sensitivity is questionable given that the chemical inhibition of these genes may likely sensitize both normal and cancer cells to PARP inhibitors. Therefore, there is an urgent need to explore additional synthetic lethal targets for PARP inhibitors.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-directed Cas9-mediated endonuclease activity can disrupt specific genetic sequences in the genome and provide a means to perform loss-of-function genetic screens (Koike-Yusa, Li et al. 2014, Shalem, Sanjana et al. 2014, Wang, Wei et al. 2014). When combined with sub-lethal doses of a PARP inhibitor, this approach may allow for the identification of genetic factors that, when disrupted, contribute to PARP inhibitor sensitivity or resistance. Unlike RNAi approaches, the CRISPR/Cas9 system provides a more thorough depletion of target gene expression with less off-target effects when the guide RNA (gRNA) is appropriately designed (Hart, Chandrashekhar et al. 2015, Munoz, Cassiani et al. 2016, Fei, Chen et al. 2017).

Therefore, we used the CRISPR/Cas9 system to perform a genome-scale loss-of-function screen to identify modifiers of olaparib sensitivity in cancer cells. From this screen, we identified *C12ofr5*, a gene that encodes a metabolic regulator, *i.e.,* TP53-induced glycolysis and apoptosis regulator (TIGAR), as a candidate that modifies olaparib sensitivity in cancer cells. We found that downregulation of TIGAR results in the enhanced cytotoxic effect of olaparib. Our results indicate that TIGAR knockdown produces two complementary effects. Together, these effects enhance sensitivity to olaparib: (1) TIGAR knockdown inhibits the pentose phosphate pathway (PPP),

resulting in an increase in intracellular reactive oxygen species (ROS) that enhances DNA damage upon olaparib treatment, and (2) TIGAR knockdown induces *"BRCAness"* by downregulation of *BRCA1* and the Fanconi anemia (FA) pathway. Finally, relevant to the therapeutic effect, TIGAR knockdown inhibits cell growth by the induction of cellular senescence. While the first two effects provide mechanisms for how TIGAR downregulation sensitizes cancer cells to olaparib, the latter reveals TIGAR as a promising strategy to prevent cancer progression. Most importantly, these *in vitro* findings were further supported by data from The Cancer Genome Atlas (TCGA). These data show that TIGAR is amplified in different cancer types, including ovarian cancer and a higher expression of TIGAR is associated with the poor overall survival of patients with high-grade serous ovarian cancer. Collectively, these results indicate that TIGAR modifies the cellular response to PARP inhibitors in cancer cells and represents a therapeutic target for developing cancer therapies.

4.3 Materials and Methods

Cell lines and cell culture

A2780 cells and ES-2 cells were maintained in MCDB105 and M199 (1:1) (Sigma, USA) containing 5% FBS (Sigma). OV90 cells were maintained in MCDB105 and M199 (1:1) with 15% FBS. OVCA420* cells were cultured in DMEM (Sigma and Caisson Labs, USA) supplemented with 10% FBS. All the media were supplemented with 100 units/mL penicillin and 100 µg/mL streptomycin. All cell lines were subjected to cell line identity confirmation. A2780-Cas9 stable cells were established by transducing A2780 cells with pLenti-cas9 followed by selection with 400 ug/mL blasticidin for 2 weeks.

Pooled Genome-Wide CRISPR Screen

Genome-scale CRISPR knock-out (GeCKO) v2.0 pooled libraries (Two-vector system) were purchased from Addgene. GeCKO library B was amplified and prepared as previously described (Sanjana, Shalem et al. 2014, Shalem, Sanjana et al. 2014). A2780 cells were infected with lentiviral particles of lenti-Cas9 at a multiplicity of infection (MOI) of ~1.0 followed by selection with blasticidin for 2 weeks to obtain the stable cell line expressing Cas9. The stable cells were then infected with the amplified lentiviral library B at an MOI of ~0.3. 3X107 cells were used and seeded in 100 mm dishes with 3×106 cells per dish. Puromycin was added the second day after lentiviral library infection and kept for 7 days. After selection, surviving cells were pooled together and split into three groups in duplicates and treated with DMSO, 5 μM or 10 μM olaparib for one week. For each treatment, 3×10^7 cells were used per replicate. Genomic DNA was extracted, and PCR was performed to prepare sequencing library as described before (Shalem, Sanjana et al. 2014). The library was sequenced using a HiSeq 2500 (Illumina). Data analysis was performed by MAGeCK tool (Li, Xu et al. 2014).

Lentiviral particle production

Viral particles were produced by transient transfection of specific plasmids (GeCKO library B, lentiCas9-Blast, individual shRNA expressing plasmids) with psPAX2 and pMD2.G (Addgene) into HEK293T cells using Lipofectamine 2000 (Life Technologies). Media was collected 48 hours after transfection and centrifuged at 2,000 rpm at 4 °C for 10 min to pellet cell debris. The supernatant was filtered through a 0.45 μm low protein binding membrane (Millipore Steriflip HV/PVDF), and aliquots were stored at -80 °C.

Antibodies and Chemicals

Mouse monoclonal anti-FLAG HRP-conjugated antibody (A8592) was purchased from Sigma. Rabbit polyclonal anti-TIGAR antibody (GTX110514) was from GeneTex (Irvine, CA, USA). Rabbit anti-Phospho-Histone H2A.X (Ser139) antibody (#2577) was purchased from Cell Signaling Technologies (Danvers, MA, USA). Rabbit polyclonal anti-BRCA1 antibody (C-20, sc-642) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal anti-beta actin antibody (A1978) was from Sigma-Aldrich (St Louis, MO, USA). For secondary antibodies, horse anti-mouse IgG-HRP antibody (7076S) was purchased from Cell Signaling Technologies, and goat anti-rabbit IgG-HRP antibody (sc-2030) was from Santa Cruz Biotechnology.

Olaparib (AZD2281, Ku-0059436) was purchased from Selleckchem. Olaparib stock solutions were made with DMSO at 50 mM and stored at -80°C. X-gal was purchased from Gold Biotechnology (St. Louis, MO), dissolved at 20 mg/mL in dimethylformamide, and stored at -20 °C in dark.

Immunoblotting

Cells were washed at least twice with PBS at the end of treatments if applicable and then lysed with an appropriate volume of 1X electrophoresis sample buffer (Bio-Rad Laboratories, CA, USA) with 5% β-mercaptoethanol (Sigma-Aldrich). The cell lysates were heated to 95 °C for 5 minutes before using. Immunoblotting procedures were performed as previously described (Zhang, Cheng et al. 2014). Equal amounts of total proteins were loaded for SDS-PAGE and transferred onto PVDF membranes (GE healthcare).

siRNA transfection

Gene-specific siRNAs and scrambled negative control siRNAs were synthesized by Integrated DNA Technologies (IDT, Coralville, IA, USA). 3.5×10^5 cells/well were seeded in 6-well plates and incubated at 37 °C overnight. Next day, 20 nanomolar of each siRNA was transfected into the cells with Oligofectamine Transfection Reagent (12252011, Invitrogen) according to manufacturer's instructions. Culture media was added 6 to 8 hours after transfection without washing cells. 24 to 48 hours after transfection, the transfected cells were trypsinized and seeded on 96-well plates (for cytotoxicity assay) or 6-well plates (for colony formation assay). Drugs were added approximately 12 hours after seeding. For cytotoxicity assay, cells were incubated with drug for 3 days before measurement of cell viability using sulforhodamine B assay. For colony formation assay, cells were exposed to drugs for 3 days and then changed to fresh media without drug for at least 10 days until colonies were observed and stained with sulforhodamine B for imaging. To check the downregulation of gene expression, the transfected cells were collected to extract total RNA for qRT-PCR or proteins for the Western blot analysis three days after transfection. The sequences of siRNAs used in the study are shown in Table 4.3.1.

Table 4.3.1

Table 4.3.1: DsiRNA sequences for candidate genes.

siRNA	Sense Strand	Antisense Strand
C12orf5 siRNA #1	rGrGrArCrArArCrCrArUrArUrArG rArArUrUrArArCrUrUAT	rArUrArArGrUrUrArArUrUrCrUrA rUrArUrGrGrUrUrGrUrCrCrArU
C12orf5 siRNA #2	rGrCrArArArGrArUrArUrGrArCrG rGrUrArArArGrUrArUGA	rUrCrArUrArCrUrUrUrArCrCrGrU rCrArUrArUrCrUrUrUrGrCrArA
PARP1 siRNA #1	rCrCrArArArGrGrArArGrGrArArC rGrCrUrArArCrArArUTT	rArArArUrUrGrUrUrArGrCrGrUrU rCrCrUrUrCrCrUrUrUrGrGrUrC
PARP1 siRNA #2	rArGrUrArUrUrGrUrUrArArGrArArC rArCrUrCrArUrGrCrAAC	GrUrUrGrCrArUrGrArGrUrGrUrU rCrUrUrArArCrArUrArCrUrUrC
NME2 siRNA #1	rCrUrGrGrGrUrCrUrArUrGrArArUr ArArGrArGrGrUrGrGAC	rGrUrCrCrArCrCrUrCrUrUrArUrU rCrArUrArGrArCrCrCrArGrUrC
NME2 siRNA #2	rArGrArCrCrArArUrCrCrArGrCrA rGrArUrUrCrArArArGCC	rGrGrCrUrUrUrGrArArUrCrUrGrC rUrGrGrArUrUrGrGrUrCrUrCrC
OR5J2 siRNA #1	rGrGrArUrCrArUrGrGrArUrArG rGrUrGrGrArArUrArGrUTA	rUrArArCrUrArUrUrCrCrArCrCrU rArUrCrCrArUrGrArUrCrCrUrG
OR5J2 siRNA #2	rUrCrUrArUrGrUrUrCrUrArUrArCrGr CrUrArGrGrGrArUTC	rGrArArUrCrCrCrUrArGrCrGrUrA rUrArGrArArCrArUrArGrArArA
IL21 siRNA #1	rCrCrArCrCrCrArArArGrArArUrUrCrC rUrArGrArArArGAT	rArUrCrUrUrUrCrUrArGrGrArArU rUrCrUrUrUrGrGrGrUrGrGrUrU
IL21 siRNA #2	rGrCrArGrUrUrGrGrArCrArCrU rArUrGrUrUrArCrArUrACT	rArGrUrArUrGrUrArArCrArUrArGrU rGrUrCrCrArArCrUrGrCrArA
scr siRNA	rCrUrUrCrCrUrCrUrCrUrUrUrCrUrCrU rCrCrCrUrUrGrUGA	rUrCrArCrArArGrGrGrArGrArGrArA rArGrArGrArGrGrArArGrGrA

qRT-PCR

The total RNA was extracted with the Trizol reagent (15596-028, Invitrogen) according to the manufacturer's manual. The cDNA was synthesized using SuperScript II reverse transcriptase (180604014, Invitrogen) with 1 μg of total RNA in a 20 μL reaction. The resulting cDNA was diluted 1:20 in nuclease-free water and 1 μL was used per qPCR reaction in triplicates. qPCR was carried out using Power SYBR Green PCR Master Mix (4367659, Thermo Fisher Scientific) on a CFX384 Real-Time PCR Detection System (Bio-Rad) including a non-template negative control. Amplification of GAPDH or 18S rRNA was used to normalize the level of mRNA expression. Primers used in the assays are shown in Table 4.3.2.

shRNAs transduction

shRNAs for C12orf5 were purchased from Sigma (SHCLNG-NM_020375). Lentiviral particles for shRNAs were produced as described above. Forty-eight hours after transfection, viral supernatant was collected and transduced into OVCA420* or OV90 cells. Experiments were conducted 2 days after viral transduction.

Cytotoxicity assay using Sulforhodamine B (SRB)

SRB assays were performed as previously described (Vichai and Kirtikara 2006) (Bastola, Neums et al. 2016) with a few modifications shown below. 3,000 cells/well were seeded in 96-well plates and treated with drugs after 12 hours of seeding. Cells were incubated for another 3 days. Dose-response curves and IC_{50} were calculated for each drug using GraphPad Prism (ver. 6) employing four parameters. All curves were constrained to 100% at on top.

Table 4.3.2

Table 4.3.2: qRT-PCR primer sequences.

Gene	Forward	Reverse
C12orf5	5′GGAAGAGTGCCCTGTGTTTAC	5′AGTTGCTTGGAGATCCTTGG
PARP1	5′AGAGAAAAGGCGATGAGGTG	5′TTAGCTCGTCCTTGATGTTCC
NME2	5′CGAGCAGAAGGGATTGGC	5′TTCATGTACTTCACCAGCCC
OR5J2	5′CAGCATTTGTGTTTCGGAGTG	5′AGCAAGGGACTCACAATGG
IL21	5’ATCAAGCTCCCAAGGTCAAG	5’AGCTGACCACTCACAGTTTG
GGT6	5’CTGAGACACAGCCGGAAAG	5’CCTCCTCCACTTCCTCCCTC
NBPF9	5’TTGAATGAGCATCTCCAGGC	5’TCTTCGTCATTTTCTGGGCTG
PA2G4	5’TTGTGGTTGATGTAGCTCAGG	3’GCAACTTTGTTCCAGGCTTC
PIK3C2G	5’GCAAATTTACTGGCGTGGAC	5’GACTTACATCCCACACTCCTG
BRCA1	5’TAATGCTATGCAGAAAATCTTAGAG	5’TACTTTCTTGTAGGCTCCTTTTGG
CCNE2	5’CTGCCTTGTGCCATTTTACC	5’GTCTTCAGCTTCACTGGACTAG
BLM	5’TGCTCTTGCTTACCATGCTG	5’GAATCACAAATCGCACGTCC
ASF1B	5’CCTTTCCACAGCCCCTTC	5’AAATTCCTCACTCTCAGCCG
CDC45	5’GGTTCAAGCACAAGTTTCTGG	5’GTACAGCTTGTCCAGGTTACTC
ECSO2	5’CTGTGGGATAAGTAGAATCTGGG	5’GGTGTTGGGTCAGAAAATGC
MAD2L1	5’GACAGATCACAGCTACGGTG	5’GGCGGACTTCCTCAGAATTG
MCM10	5’AACCAGCCATCAAGTCCATC	5’TGGGCTCTCAACTTCACTTG
MKI67	5’AAAAGAATTGAACCTGCGGAAG	5’AGTCTTATTTTGGCGTCTGGAG
MYBL2	5’TGTGGATGAGGATGTGAAGC	5’TGAGGCTGGAAGAGTTTGAAG
PCNA	5’CCGAAACCAGCTAGACTTTCC	5’GATGAGGTCCTTGAGTGCC
TCF19	5’TGGCCTCATCTCTGGGATC	5’TCTCCATCACTCAATTCCAGC
UBE2C	5’TCTGGCGATAAAGGGATTTCTG	5’CTTGAGTTTCTCTGGGACCG
GAPDH	5’GAAACTGTGGCGTGATGGC	5’CACCACTGACACGTTGGCAG
18S rRNA	5’GCCCGAAGCGTTTACTTTGA	5’TCCATTATTCCTAGCTGCGGTATC

Colony formation assay

500 to 1,000 cells were seeded in 6-well plates and treated with drugs after 12 hours of seeding. Cells were incubated for another 3 days before changing to fresh media. The medium was changed every 2 to 3 days to allow colonies to form. At the end of experiments, colonies were stained with SRB and imaged with Molecular Imager GelDoc XR System (Bio-Rad). The stained SRB was solubilized in Tris buffer and measured with a plate reader. The analysis of colonies was performed in GraphPad Prism (ver. 6).

Analysis of apoptosis with Annexin V/PI staining

Cells were transfected with scrambled siRNA or C12orf5 siRNA and reseeded into 6-well plates after 24 hours of transfection, followed by treatment with Vehicle, 10 μM or 20 μM of olaparib for additional 48 hours. Cells were then subject to Annexin V/PI staining following manufacturer's instruction (#640906, Biolegend). Briefly, cells were washed twice with PBS and stained with Annexin V and PI solution for 15 minutes at room temperature in the dark before analysis by flow cytometry.

Measurement of Intracellular Reactive Oxygen Species (ROS)

Intracellular ROS levels were measured by the oxidant-sensitive fluorescent probe 5-(and-6)-chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA, Molecular Probes). Forty-eight hours after transfection with scramble siRNA or C12orf5 siRNA, cells were trypsinized, washed twice with PBS, and incubated with 5 μM CM-H2DCFDA in PBS at 37 °C for 30 minutes. Propidium iodide (PI) was added 10 minutes before flow analysis to exclude dead cells from ROS analysis.

Neutral comet assay

Neutral comet assay was performed according to Tregvigen instructions for Comet Assay with some modifications. Briefly, 48 hours after siRNA transfection, cells were treated with vehicle, 5 μM or 20 μM olaparib for 24 hours. Cell suspension in PBS with $1x10^5$ cells/mL was mixed with molten LMA agarose (Trevigen) at a ratio of 1:10 and transferred onto Comet Slide (Trevigen). After cooling slides at 4 °C for 10 minutes, slides were immersed in the Lysis solution (Trevigen, 4250-050-01) at 4 °C for 1 hour and immersed in 50 mL of 1X Neutral Electrophoresis Buffer (0.5 mM Tris Base and 1.5 mM Sodium Acetate) for 30 minutes at 4 °C. One hour of electrophoresis was performed at 4°C using Wide Mini-Sub Cell GT Horizontal Electrophoresis System (Bio-Rad) at 17 volts. Slides were then immersed in the DNA Precipitation Solution (1M NH4Ac and 82.27% ethanol) for 30 minutes at room temperature followed by 30 minutes incubation in 70% ethanol. Then slides were dried at 37 °C for 10-15 minute stained with SYBR Green I and imaged using ZEISS fluorescent microscope (10X). Comets were analyzed using a free comet assay software from Casp Lab (1.2.3beta2 version).

Cell cycle analysis

Cells were transfected with scrambled (scr) siRNA or C12orf5 siRNA and reseeded into 6-well plates after 24 hours of transfection, followed by treatment with different concentrations of olaparib for additional 24, 48 or 72 hours. After treatment, cells were washed twice with 1X PBS and trypsinized before centrifuging at 1,000 rpm at 4 ℃ for 10 minutes in 15 mL conical tubes. Cells were resuspended in 300 μL of ice-cold PBS after wash, fixed by adding 300 μL of ice-cold 95% ethanol dropwise, and kept in dark at 4 ℃ overnight. The next day, cells were washed twice with ice-cold PBS and incubated in RNase A solution (1 mg/mL) at 37 °C for 15 minutes. Cells were then stained with 50 μg/mL propidium iodide solution in dark at room temperature for 15 minutes before flow cytometry analysis.

Senescence-Associated β-Galactosidase Assay (SA-β-Gal)

Two days after transducing with individual C12orf5 shRNAs, cells were trypsinized and reseeded in 6-well plates at the plating density of 1×10^5 cells per well and incubated at 37 °C for 24 hours. SA-β-Gal staining was performed as described before for adherent cultured cells (Itahana, Campisi et al. 2007). Briefly, cells were washed twice with 1X PBS, fixed with freshly prepared 3.7% formaldehyde in PBS for 5 minutes at room temperature, and washed twice with PBS. Cells were incubated in 2 mL of X-gal staining solution [1 mg/mL of X-gal, 40 mM citric acid/sodium phosphate buffer (pH 6.0), 5 mM potassium ferricyanide (Sigma, St. Louis, MO), 5 mM potassium ferrocyanide (Sigma), 150 mM NaCl, and 2 mM $MgCl_2$] at 37 °C for 16 hours before taking images with 10X objective under Leica DMI3000 B Inverted Phase Contrast Microscope. SA-β-Gal stain-positive cells were quantified in Image J software (NIH).

Spheroid formation assay

OVCA420* cells stably expressing inducible C12orf5 shRNAs (TRIPZ inducible lentiviral shRNAs were purchased from Dharmacon, US) were established by selection with puromycin after viral transduction. Stable cells were treated with 1 μg/mL doxycycline for 72 hours to induce expression of shRNAs. Then 3,000 cells/well were seeded into 96 well plates (plates were pre-coated with 100 μL 1.5% (wt. /vol) agarose) and treated with vehicle or different concentrations of olaparib for 10 days. At the end of the experiment, pictures of spheroid were taken under a microscope and cell viability was measured with CellTiter-Glo Luminescent Cell Viability Assay (Promega Corporation).

RNA-sequencing analysis

Total RNA was extracted 54 hours after cells were transduced with TIGAR shRNA viral supernatant. One µg of total RNA was used to synthesize cDNA and sequencing library was prepared using Illumina TruSeq RNA Sample Preparation Kit v2 following manufacturer's instructions. Cells transduced with non-targeting shRNA serves as a control. RNA-seq libraries were generated from three replicates from the control and experimental groups. Next-generation sequencing was performed with NextSeq 500 at the Oklahoma Medical Research Foundation (OMRF) Clinical Genomics Center (OK, USA). FASTQ sequences were mapped to the human reference genome (hg19) using STAR aligner (Dobin, Davis et al. 2013). Read count for quantitation of gene expression was performed by RSEM (Li and Dewey 2011). Differentially expressed genes in TIGAR knockdown cells were determined by DESeq2 (Love, Huber et al. 2014). Differentially expressed genes were further analyzed with Metascape (Tripathi, Pohl et al. 2015).

Statistical analysis

All data were analyzed using GraphPad Prism (ver. 6) unless otherwise indicated. Results were expressed as the mean ± standard error of the mean (SEM.) or standard deviation of the mean (S.D). Differences between treatment regimens were analyzed by one-way ANOVA or two-tailed Student's t-test. $p<0.05$ was considered to be statistically significant. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

4.4 Results

4.4.1 Genome-scale CRISPR knockout screen identifies *C12orf5* (*TIGAR*) as a modifier of olaparib sensitivity

To identify modifiers of olaparib sensitivity, we first established a cell line that stably expressed Cas9 endonuclease (A2780-Cas9) (Figure 4.4.1.A-B) by transducing the A2780 cells with lenti-Cas9 viral particles followed by two weeks of blasticidin selection. Cas9 expression in this stable cell line was confirmed by western blot (Figure 4.4.1.A). Sulforhodamine B (SRB) assay showed that Cas9 expression and blasticidin S (an antibiotic that is produced by *Streptomyces griseochromogene*) selection had a minimal effect on sensitivity to olaparib in A2780-Cas9 compared to the parental A2780 cell (Figure 4.4.1.B). The screening was performed by following the time scheme shown in Figure 4.4.1C. We transduced the Cas9-expressing cells with Genome-scale CRISPR Knock-Out Library B, which contains 58,031 gRNAs targeting 19,050 human genes with three gRNAs per gene. After seven days of puromycin selection, cells expressing gRNAs were split into duplicates and treated with DMSO or olaparib (5 μM or 10 μM). The DMSO-treated cells served as a control. One week after DMSO or olaparib treatment, the remaining cells were collected, gRNAs were amplified, and the sequence library was prepared as previously described (Sanjana, Shalem et al. 2014, Shalem, Sanjana et al. 2014). Amplicons were sequenced by Illumina next-generation sequencing. Enriched or depleted gRNAs were identified using the Model-based Analysis of Genome-wide CRISPR-Cas9 Knockout (MAGeCK) tool to compare DMSO- and olaparib-treated groups (MAGeCK) tool (Li, Xu et al. 2014). Ten top candidates were identified for enrichment (Figure 4.4.1.D-E) or depletion (Figure 4.4.1.F-G) analysis. The enrichment analysis identified candidate genes whose disruptions led to olaparib resistance, including *PARP1* (Figure 4.4.1.D-E). Thus, these genes are potential candidates to increase sensitivity to olaparib.

Figure 4.4.1

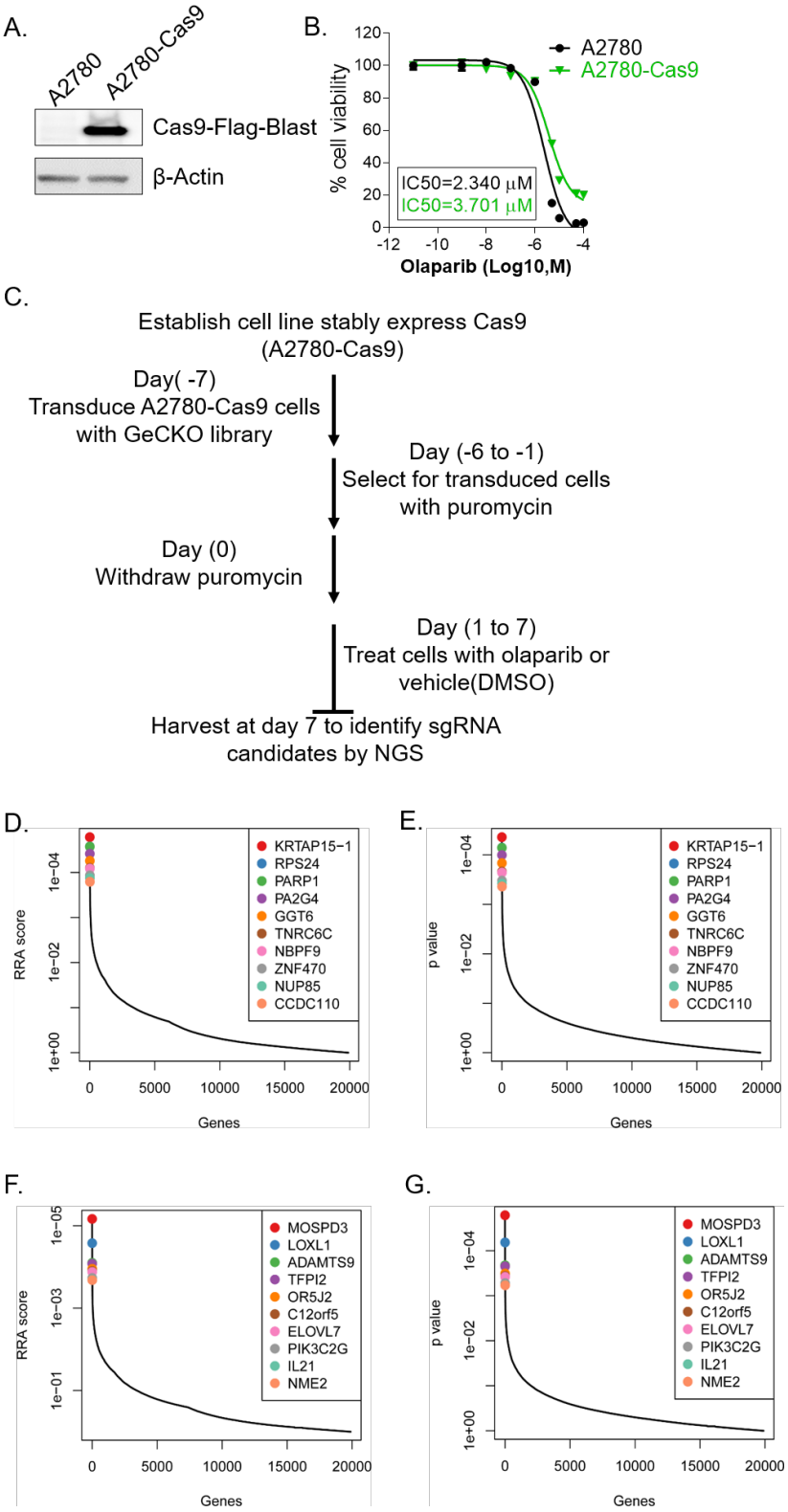

A.
A2780
A2780-Cas9
Cas9-Flag-Blast
β-Actin
B.
% cell viability
A2780
A2780-Cas9
IC50=2.340 μM
IC50=3.701 μM
Olaparib (Log10,M)
C.
Establish cell line stably express Cas9 (A2780-Cas9)
Day(-7) Transduce A2780-Cas9 cells with GeCKO library
Day (-6 to -1) Select for transduced cells with puromycin
Day (0) Withdraw puromycin
Day (1 to 7) Treat cells with olaparib or vehicle(DMSO)
Harvest at day 7 to identify sgRNA candidates by NGS
D.
RRA score
E.
p value
KRTAP15−1
RPS24
PARP1
PA2G4
GGT6
TNRC6C
NBPF9
ZNF470
NUP85
CCDC110
Genes
F.
G.
MOSPD3
LOXL1
ADAMTS9
TFPI2
OR5J2
C12orf5
ELOVL7
PIK3C2G
IL21
NME2

Figure 4.4.1: Genome-scale CRISPR knockout screening and candidate identification. A) A2780-Cas9 cells stably express Cas9 endonuclease. Flag-tagged Cas9 was detected by Western blot with anti-Flag antibody. β-actin was used as a loading control. **B)** A2780-Cas9 showed similar sensitivity to olaparib as parental A2780 cells. 3-day SRB assay was used to determine cell viability after treatment of different concentrations of olaparib. Cell survival curves were plotted in Prism 6 software. Data shown as mean ± SEM. IC_{50} was calculated in Prism 6 software. **C)** Flowchart shows time scheme of the CRISPR/Cas9 knockout screen. **D-G)** Potential dropout and enriched candidates were identified after olaparib selection. (D) and (E) show top 10 candidates which were most significantly depleted after olaparib selection. (F) and (G) show top 10 candidates which were most significantly enriched with olaparib selection. Candidates were identified with MAGeCK tool (Li, Xu et al. 2014) by comparison of olaparib treated groups with DMSO treated groups.

All three gRNAs showed consistent, dose-dependent increases in abundance following olaparib treatment (Figure 4.4.2.A). These results are consistent with a previous report indicating that *PARP1* depletion results in resistance to PARP inhibitors (Liu, Han et al. 2009, Pettitt, Rehman et al. 2013). While the enrichment of gRNAs following olaparib selection indicates that target genes contribute to olaparib sensitivity, the depletion of gRNAs following olaparib selection indicates that target genes contribute to olaparib resistance. We identified *C12orf5* (TIGAR) as one of the genes that negatively modulated olaparib sensitivity (Figure 4.4.1.F-G) because gRNAs for *C12orf5* were depleted following olaparib treatment. This suggests that disruption of the *C12orf5* gene sensitized the cells to olaparib. We also identified *PI3K3C2G* and *NME2* as other candidate genes that decreased sensitivity to olaparib (Figure 4.4.1.F-G). Next, we transiently knocked down individual genes with pooled small interfering RNAs (siRNAs) to validate the potential candidates. We selected for validation those candidates that had at least two different gRNAs that were consistently and dose-dependently enriched or depleted after olaparib treatment, including *C12orf5* and *PARP1* (Figure 4.4.2.A-B).

Figure 4.4.2

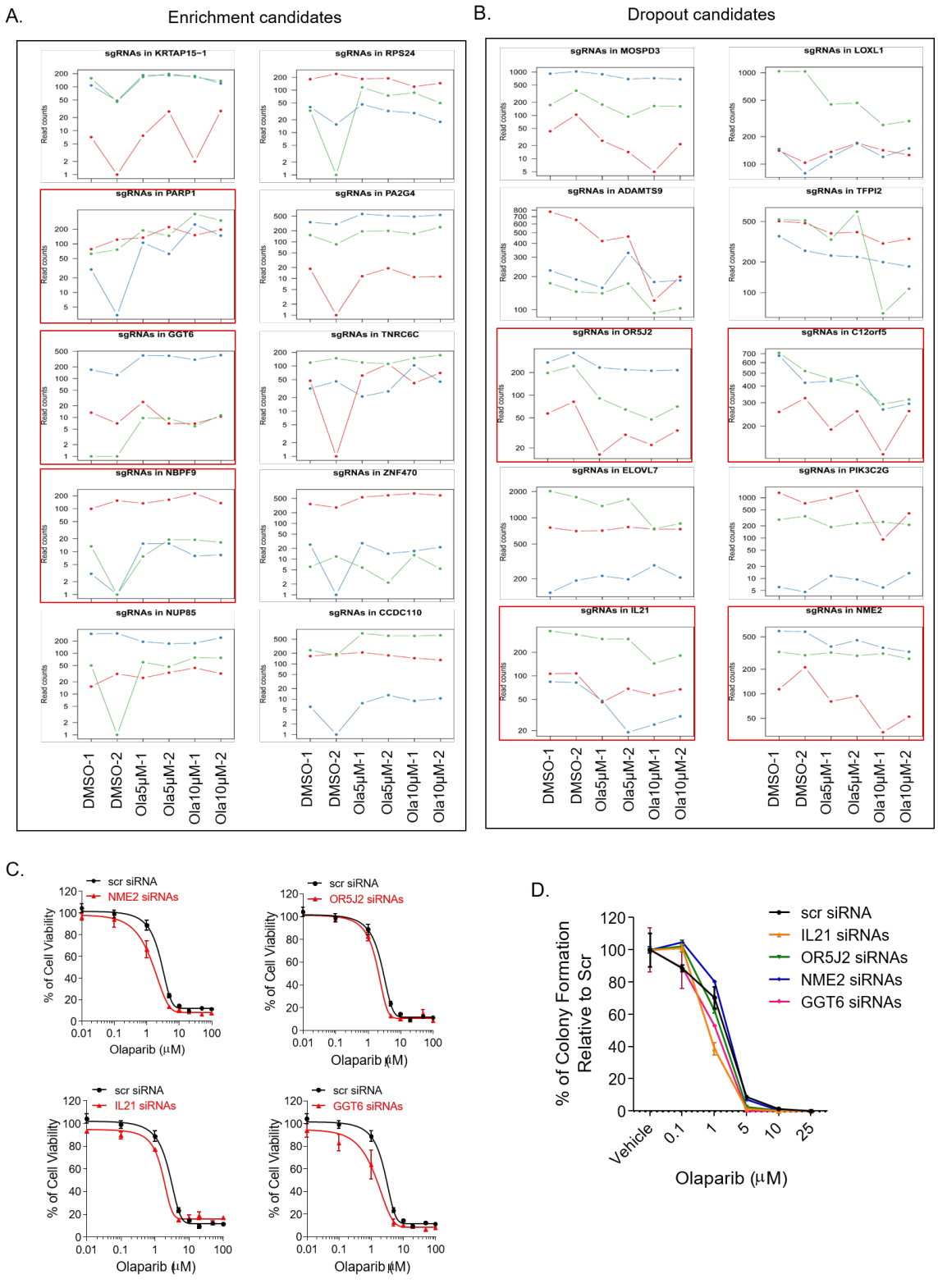

A.
Enrichment candidates
sgRNAs in KRTAP15−1
sgRNAs in RPS24
sgRNAs in PARP1
sgRNAs in PA2G4
sgRNAs in GGT6
sgRNAs in TNRC6C
sgRNAs in NBPF9
sgRNAs in ZNF470
sgRNAs in NUP85
sgRNAs in CCDC110
B.
Dropout candidates
sgRNAs in MOSPD3
sgRNAs in LOXL1
sgRNAs in ADAMTS9
sgRNAs in TFPI2
sgRNAs in OR5J2
sgRNAs in C12orf5
sgRNAs in ELOVL7
sgRNAs in PIK3C2G
sgRNAs in IL21
sgRNAs in NME2
Read counts
DMSO-1
DMSO-2
Ola5μM-1
Ola5μM-2
Ola10μM-1
Ola10μM-2
C.
scr siRNA
NME2 siRNAs
OR5J2 siRNAs
IL21 siRNAs
GGT6 siRNAs
% of Cell Viability
Olaparib (μM)
D.
% of Colony Formation Relative to Scr
scr siRNA
IL21 siRNAs
OR5J2 siRNAs
NME2 siRNAs
GGT6 siRNAs
Vehicle
Olaparib (μM)

Figure 4.4.2: Validation of potential candidates. A) Profiles of dropout candidate. 3 different gRNAs for each candidate were shown. Each dot represents corresponding treatment condition: DMSO-1, DMSO-2, olaparib 5 μM-1, olaparib 5 μM-2, olaparib 10 μM-1 and olaparib 10 μM-2. gRNA read count decreases with olaparib treatment in a dose-dependent manner, such as *C12orf5*. **B)** Profiles of enrichment candidates. gRNA read count increases with olaparib treatment in a dose-dependent manner, such as *PARP1*. Candidates with red boxes were selected for further validation with pooled siRNAs. **C)** SRB cell viability assay indicates no change of sensitivity to olaparib after transfection of pooled siRNAs of indicated potential candidates. 48 hours after siRNA transfection, cells were treated with olaparib for 3 days. Data are shown as mean ± SEM. Results are representatives from one experiment with triplicates. **D)** Colony formation assay also indicates no change in olaparib sensitivity after transfection with pooled siRNAs for each candidate. 48 hours after siRNA transfection, cells were treated with olaparib for 3 days and kept to allow colonies to grow for 2 weeks before colony. Data are shown as mean ± SEM. Results are representatives of experiments with duplicates.

4.4.2 *TIGAR* knockdown by siRNA enhances sensitivity to olaparib in cancer cells

To validate the selected candidates, we used pooled siRNAs with two different siRNAs for each gene to downregulate their expression in the A2780 cells from the initial screen. Forty-eight hours after siRNA transfection, we performed SRB and colony formation assays to assess the sensitivity to olaparib (Figure 4.4.3.A-C, Figure 4.4.2C-D). The results showed that a transient knockdown of TIGAR (Figure 4.4.3.B) led to an approximate 5-fold decrease in IC_{50} of olaparib in the A2780 cells (Figure 4.4.3.A). Clonogenic survival analysis also indicated a decrease in colony formation in TIGAR knockdown cells when combined with various concentrations of olaparib (Figure 4.4.3.C). Similar results were obtained with SRB assay in two additional cancer cell lines that were less sensitive to olaparib (Figure 4.4.3.E-F), suggesting that downregulation of TIGAR sensitized these cells to olaparib. Meanwhile, we observed that by itself TIGAR knockdown significantly decreased colony formation (Figure 4.4.3.D). Similar results were also observed in cancer cell OVCA420* (Figure 4.4.3.G-H). These observations are consistent with a previous report on glioblastoma cells (Pena-Rico, Calvo-Vidal et al. 2011). These results suggest that TIGAR depletion attenuates the growth of cancer cells and enhances sensitivity to olaparib.

Figure 4.4.3

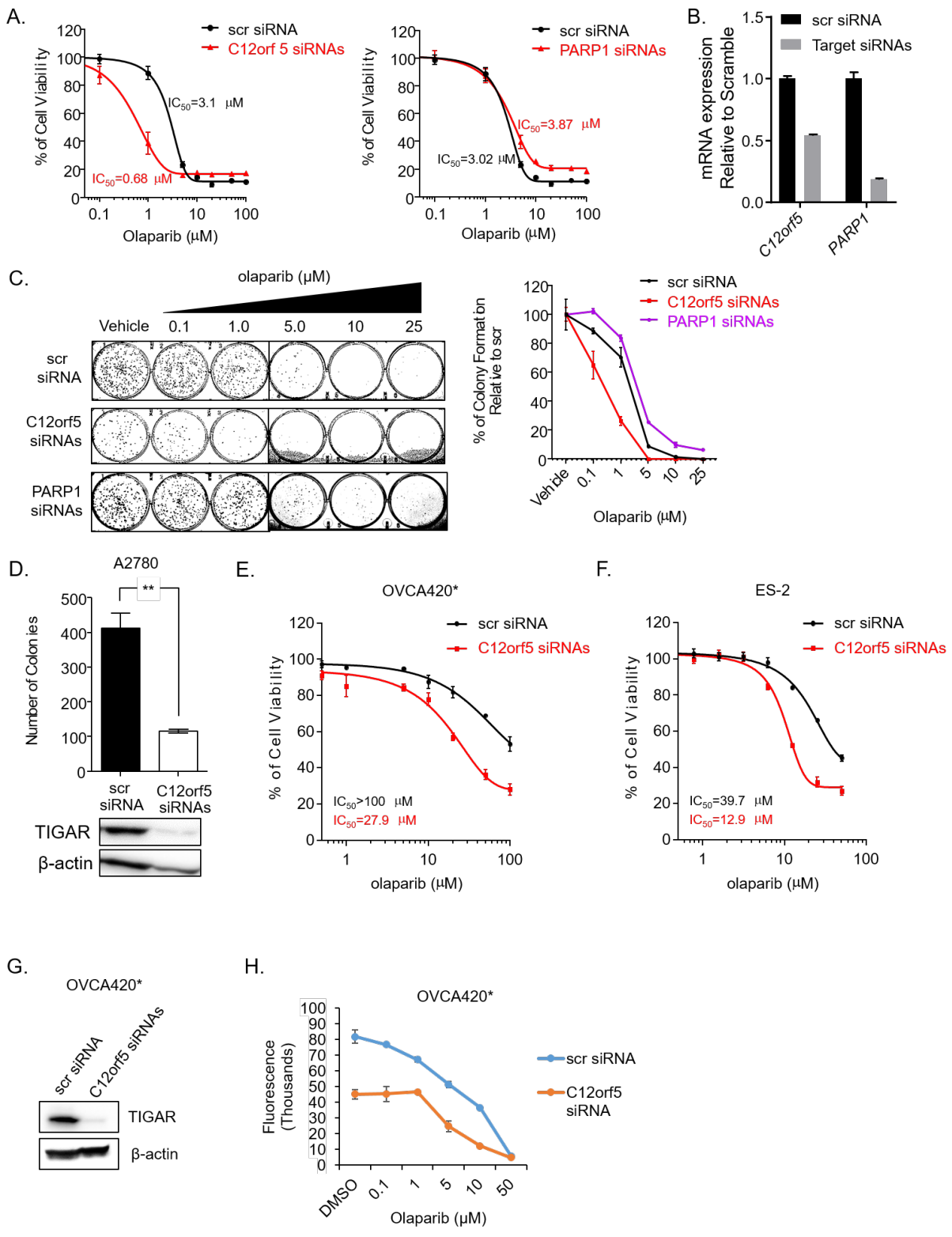

A.
scr siRNA
C12orf 5 siRNAs
% of Cell Viability
IC50=3.1 μM
IC50=0.68 μM
Olaparib (μM)
scr siRNA
PARP1 siRNAs
IC50=3.87 μM
IC50=3.02 μM
Olaparib (μM)
B.
scr siRNA
Target siRNAs
mRNA expression Relative to Scramble
C12orf5
PARP1
C.
olaparib (μM)
Vehicle
0.1
1.0
5.0
10
25
scr siRNA
C12orf5 siRNAs
PARP1 siRNAs
% of Colony Formation Relative to scr
scr siRNA
C12orf5 siRNAs
PARP1 siRNAs
Olaparib (μM)
D.
A2780
**
Number of Colonies
scr siRNA
C12orf5 siRNAs
TIGAR
β-actin
E.
OVCA420*
scr siRNA
C12orf5 siRNAs
% of Cell Viability
IC50>100 μM
IC50=27.9 μM
olaparib (μM)
F.
ES-2
scr siRNA
C12orf5 siRNAs
% of Cell Viability
IC50=39.7 μM
IC50=12.9 μM
olaparib (μM)
G.
OVCA420*
scr siRNA
C12orf5 siRNAs
TIGAR
β-actin
H.
OVCA420*
Fluorescence (Thousands)
scr siRNA
C12orf5 siRNA
DMSO
Olaparib (μM)

Figure 4.4.3: *TIGAR* knockdown with siRNAs sensitizes cancer cells to olaparib. A) *TIGAR* knockdown results in a decrease in IC_{50} of olaparib, while *PARP1* knockdown results in an increase in IC_{50} of olaparib in A2780 cells. 3-day SRB cell viability assay was performed 48 hour after pooled siRNAs transfection in A2780 cells. Cell survival curves were plotted using Prism 6 software with IC_{50} indicated. **B)** Real-time PCR to validate knockdown of *TIGAR* and *PARP1* with corresponding pooled siRNAs. Total RNA was extracted 72 hours after siRNA transfection and used for qRT-PCR. **C)** Colony formation assay shows that *TIGAR* knockdown enhanced decrease of colony formation with olaparib treatment and *PARP1* knockdown attenuated it. Left: Representative images of colonies. Right: Quantification of colonies. The clonogenic assay was performed 72 hours after siRNA transfection. Cells were exposed to vehicle or different concentrations of olaparib for 3 days. Data are shown as mean ± SEM. **D)** *TIGAR* knockdown results in decreased colony formation. Colony quantification for scr siRNA and *TIGAR (C12orf5)* siRNAs transfected cells. Data shown as mean ± SEM. Statistics analysis was done with Student's t-test. **$p<0.01$. **E-F)** *TIGAR* siRNA knockdown increases sensitivity to olaparib in OVCA420* cell (E) and ES-2 cell (F) respectively. SRB cell viability assay was performed and cell survival curves were generated in Prism 6 software with IC_{50} indicated. Data shown as mean ± SEM. **G-H)** *TIGAR* knockdown decreases cell survival in OVCA420* cell. **G**) Western blot of *TIGAR* expression 72 hours after siRNA transfection. β-actin was used as a loading control. **H**) SRB cell viability assay. 48 hours after siRNA transfection, cells were treated with olaparib for 3 days. Data are shown as mean ± SEM. A representative of experiment with triplicates is shown.

We were also able to validate the effect of *PARP1* knockdown on olaparib sensitivity. *PARP1* knockdown showed a decrease in sensitivity to olaparib in the A2780 cells (Figure 4.4.3.A-C). This is consistent with previous reports that *PARP1* depletion leads to PARP inhibitor resistance (Liu, Han et al. 2009, Pettitt, Rehman et al. 2013). However, we were unable to validate the effect of other candidate genes on olaparib sensitivity (Figure 4.4.2.C-D). It should be noted that we only observed a marginal decrease in sensitivity to olaparib with the transient knockdown of *PARP1* by siRNAs. This effect was not as dramatic as previous studies that showed a 10-fold decrease in PARP inhibitor sensitivity with the genetic disruption of *PARP1* expression (Liu, Han et al. 2009, Pettitt, Rehman et al. 2013). This difference may be the result of the incomplete knockdown of *PARP1* by siRNAs in our study. Differences in the efficiency of candidate gene suppression by the siRNAs and CRISPR could be one reason to explain our failure to validate some candidate genes from the CRISPR knockout screen.

4.4.3 *TIGAR* knockdown induces apoptosis, increases DNA damage and enhances cytotoxicity of olaparib

Next, we determined the mechanisms by which TIGAR downregulation leads to olaparib sensitivity. TIGAR shows sequence similarity to the bisphosphatase domain of fructose-2,6-bisphosphatase (F-2,6-BPase) (Bensaad, Tsuruta et al. 2006). TIGAR has been shown to negatively regulate glycolysis by lowering the intracellular levels of fructose-2,6-bisphosphate, thereby promoting the oxidative PPP shunt. The PPP is critical for the production of NADPH and ribose-5-phosphate, which is a precursor for the synthesis of nucleotides (Bensaad, Tsuruta et al. 2006, Wanka, Steinbach et al. 2012). TIGAR expression also protects cells from DNA-damaging ROS through the regeneration of reduced glutathione via NADPH and provides some levels of protection from DNA damage-induced apoptosis (Bensaad, Tsuruta et al. 2006, Bensaad, Cheung

et al. 2009, Wanka, Steinbach et al. 2012, Cheung, Athineos et al. 2013). Therefore, we tested the effect of TIGAR knockdown on apoptosis with or without olaparib treatment. With Annexin V/PI staining, we observed a dramatic increase in apoptotic cells after TIGAR knockdown with pooled siRNAs in two different cells (Figure 4.4.4.A-C). The increase in apoptosis was further enhanced when combined with olaparib, suggesting that TIGAR knockdown can potentiate the cytotoxicity effects of olaparib through the increase in apoptosis.

Figure 4.4.4

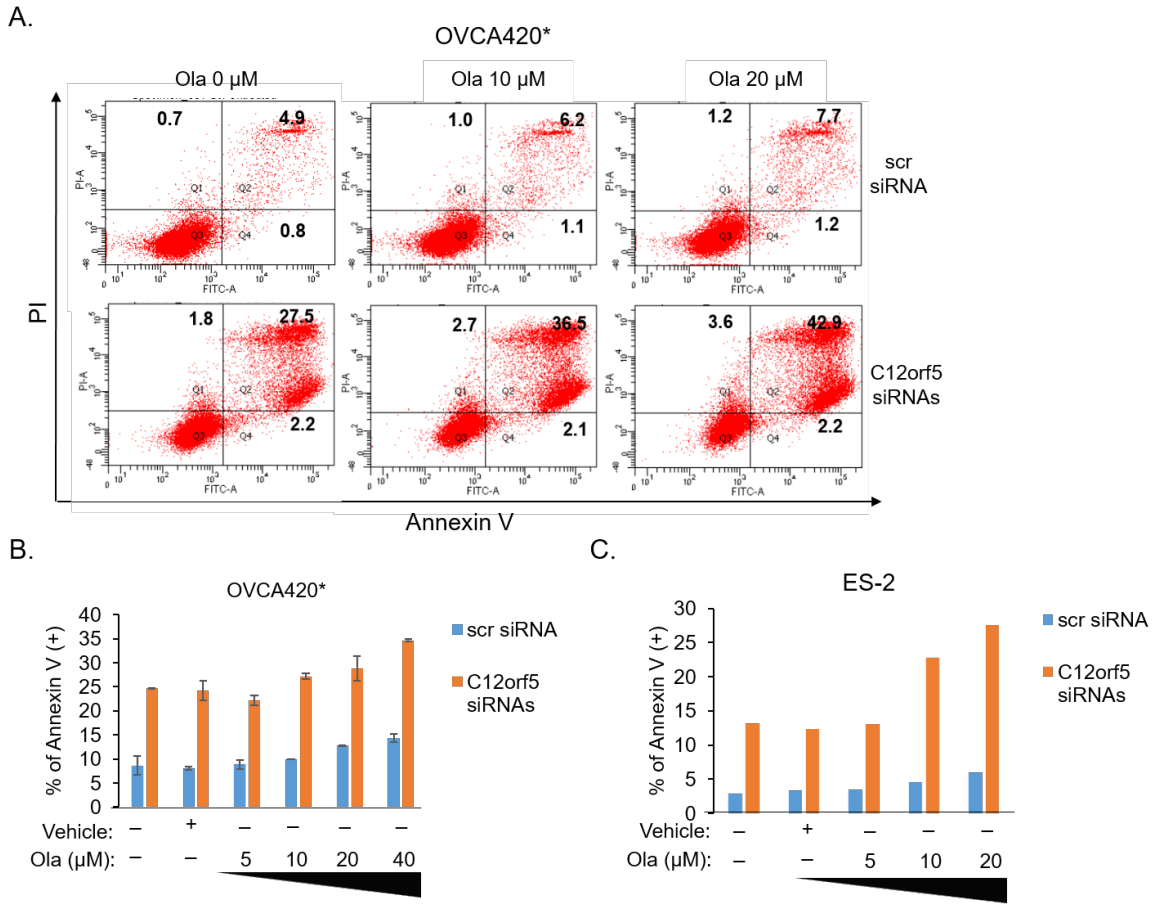


Figure 4.4.4: *TIGAR* knockdown induces apoptosis. A) *TIGAR* knockdown increases apoptosis and enhances the cytotoxicity of olaparib in OVCA420* cell. Representative flow cytometry analysis profiles of Annexin V/PI stained cells with vehicle or olaparib treatment after scrambled (scr) siRNA or *TIGAR* siRNAs transfection. 48 hours after siRNA transfection, cells were seeded for the vehicle, 10 μM or 20 μM olaparib treatment for another 48 hours before Annexin V/PI staining. **B-C)** Quantification analysis shows that *TIGAR* knockdown results in the increase in apoptosis and enhanced cytotoxicity by olaparib in OVCA420* and ES-2 cells. Annexin V (+) cells represent both early and late apoptotic cell. For OVCA420*, data are shown as mean with S.D and results shown as one representative experiment with triplicates. For ES-2, one representative experiment was shown.

Given that TIGAR enhances antioxidant NADPH regeneration by promoting the PPP, TIGAR knockdown results in increased levels of intracellular ROS as shown in previous studies (Bensaad, Tsuruta et al. 2006, Bensaad, Cheung et al. 2009). To test this possibility, we determined cellular ROS levels by H_2DCFDA dye staining. After knockdown of TIGAR with an individual siRNA or pooled siRNAs, we observed higher H_2DCFDA fluorescence compared to scrambled siRNA (scr siRNA)-transfected cells (Figure 4.4.5.A), with a 1.35-fold and 1.33-fold increase of mean fluorescence intensity in TIGAR siRNA#1 and siRNA#2 respectively, and the pooled siRNAs leads to 1.52-fold increase of mean fluorescence intensity compared to scr siRNA transfected cells. This indicates that TIGAR downregulation increased ROS levels in the cells. Consistent with increased levels of ROS, neutral comet assay showed a significant increase in DNA damage after TIGAR knockdown (Figure 4.4.5.B). Moreover, TIGAR knockdown significantly enhanced DNA damage caused by olaparib treatment. Consistent with higher levels of DNA damage, cells with TIGAR knockdown displayed higher levels of γH2AX at 24 and 48 hours following olaparib treatment than cells transfected with scr siRNA (Figure 4.4.5.C). Yu et al. (2015) have shown that TIGAR knockdown increased DNA damage by inhibiting the PPP in hepatocellular carcinoma cells (Yu, Xie et al. 2015). We supplemented cells with NADPH, ribose or N-acetyl cysteine and observed a partial decrease in γH2AX with NAC and Ribose (Figure 4.4.5.D), suggesting that enhanced DNA damage after TIGAR knockdown is at least partially due to a reduction of substrates from the PPP.

Figure 4.4.5

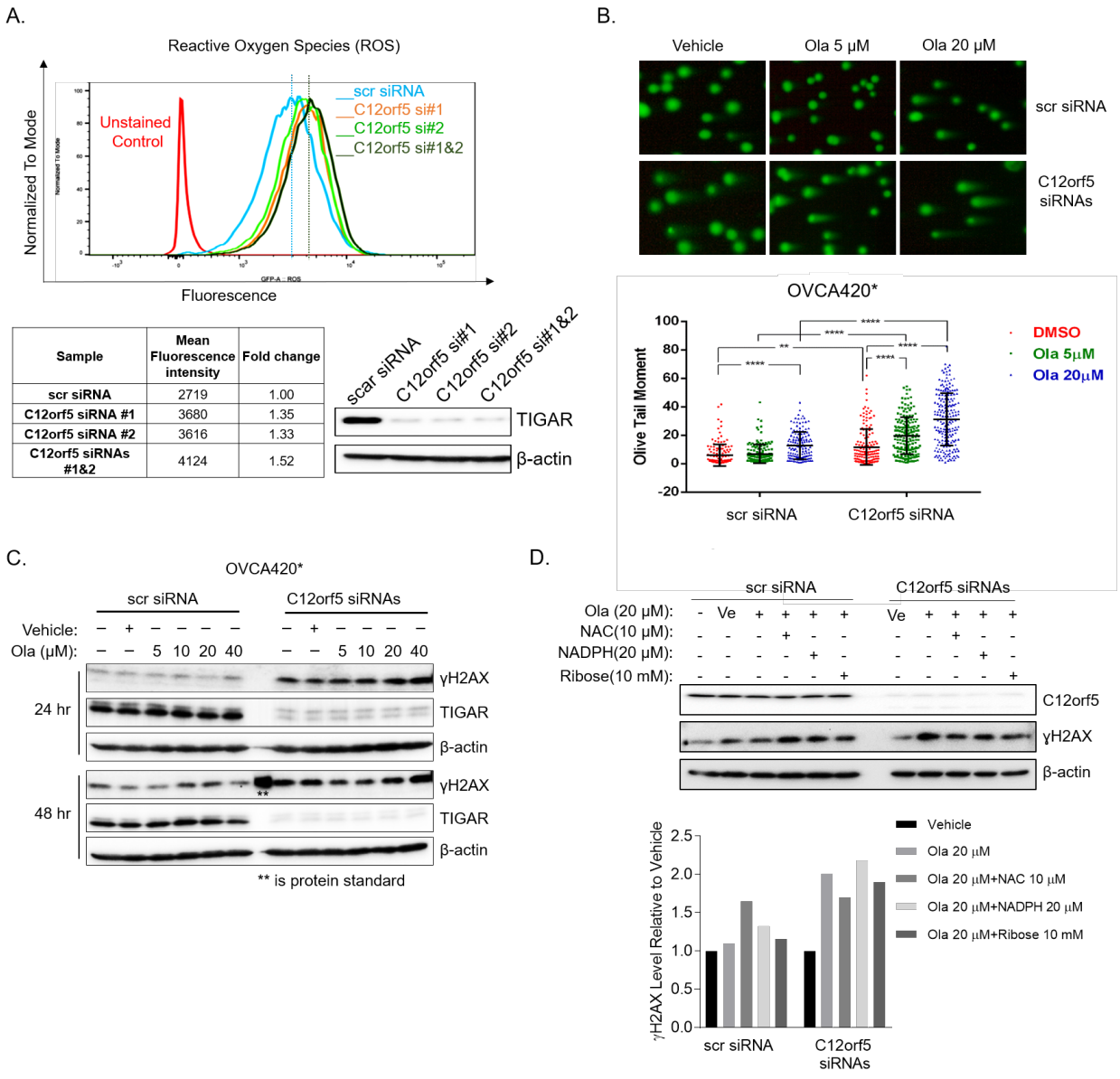


Figure 4.4.5: *TIGAR* knockdown results in an increase of DNA damage through induction of intracellular ROS. A) *TIGAR* knockdown leads to increase of intracellular ROS levels. Upper: 48 hours after siRNA transfection, cells were loaded with 5 μM H2DCFDA for 30 minutes before flow cytometry analysis. Dead cells were excluded by PI staining. Mean fluorescence intensity and fold change were shown in the chart. Western blot shows that TIGAR is downregulated with siRNAs. β-actin was used as a loading

control. **B)** *TIGAR* knockdown results in significantly increased DNA damage and further significantly enhanced with olaparib treatment. 48 hours after siRNA transfection, OVCA420* cells were treated with vehicle or olaparib for 24 hours. DNA damage was detected by Neutral Comet Assay. Representative image of each group was shown on the top. Individual comets were analyzed using Capslab free comet tail analysis software. Quantification data are shown in the graph at the bottom. Olive Tail Moment = (Tail.mean - Head.mean) X Tail%DNA/100. Each dot represents one comet. Data are shown as median with S.D. Statistics were done with One-way ANOVA analysis in Prism 6 software. **=$p<0.01$, ****=$p<0.0001$. **C)** *TIGAR* knockdown increases γH2AX level in OVCA420* cells. 48 hours after siRNA transfection, OVCA420* cells were treated with vehicle or olaparib for 24 hours or 48 hours. β-actin was used as a loading control. **D)** The increase of γH2AX level after olaparib treatment in *TIGAR* knockdown cells can be partially attenuated with NAC, NADPH or ribose. 48 hours after transfection, cells were pretreated with 10 μM NAC, 20 μM NADPH or 10 mM ribose respectively for 2 hours, followed by 24 hours treatment with vehicle or 20 μM olaparib. Result is from one experiment. Bottom bar graph shows quantification of western blots.

We also performed a cell cycle analysis in ES-2 cells with or without TIGAR siRNA knockdown and in combination with olaparib treatment. Consistent with a previous report (Farmer, McCabe et al. 2005), the 24-hour treatment of scr siRNA-transfected ES-2 cells with olaparib resulted in dose-dependent cell cycle arrest at the G_2 or M phase in cells transfected with scr siRNA, with almost 2-fold increase of G2 or M phase cells in 20 μM olaparib treated group compared to DMSO treated group (Figure 4.4.6.A). Interestingly, we observed S-phase arrest after TIGAR knockdown (Figure 4.4.6.A). TIGAR knockdown leads to 1.35-fold increase of S-phase cells in comparison with scr siRNA transfected cells. Moreover, more cells were arrested in the S-phase instead of the G_2 or M phases in TIGAR knockdown cells treated with olaparib. 39.2% of TIGAR knockdown cells were at S-phase when treated with 20 μM of olaparib versus only 22.9% of scr siRNA transfected cells. Similar increase of S-phase arrest were observed at 48 and 72 hours after olaparib treatment (Figure 4.4.6.B-D). These data are consistent with the notion that an increase in DNA damage after TIGAR knockdown requires more time to resolve DNA damage during the S-phase in TIGAR knockdown cells. In addition, TIGAR knockdown may also affect the expression of genes associated with S-phase progression and altered cell cycle-related gene expression may also contribute to the delay in S-phase progression.

Figure 4.4.6

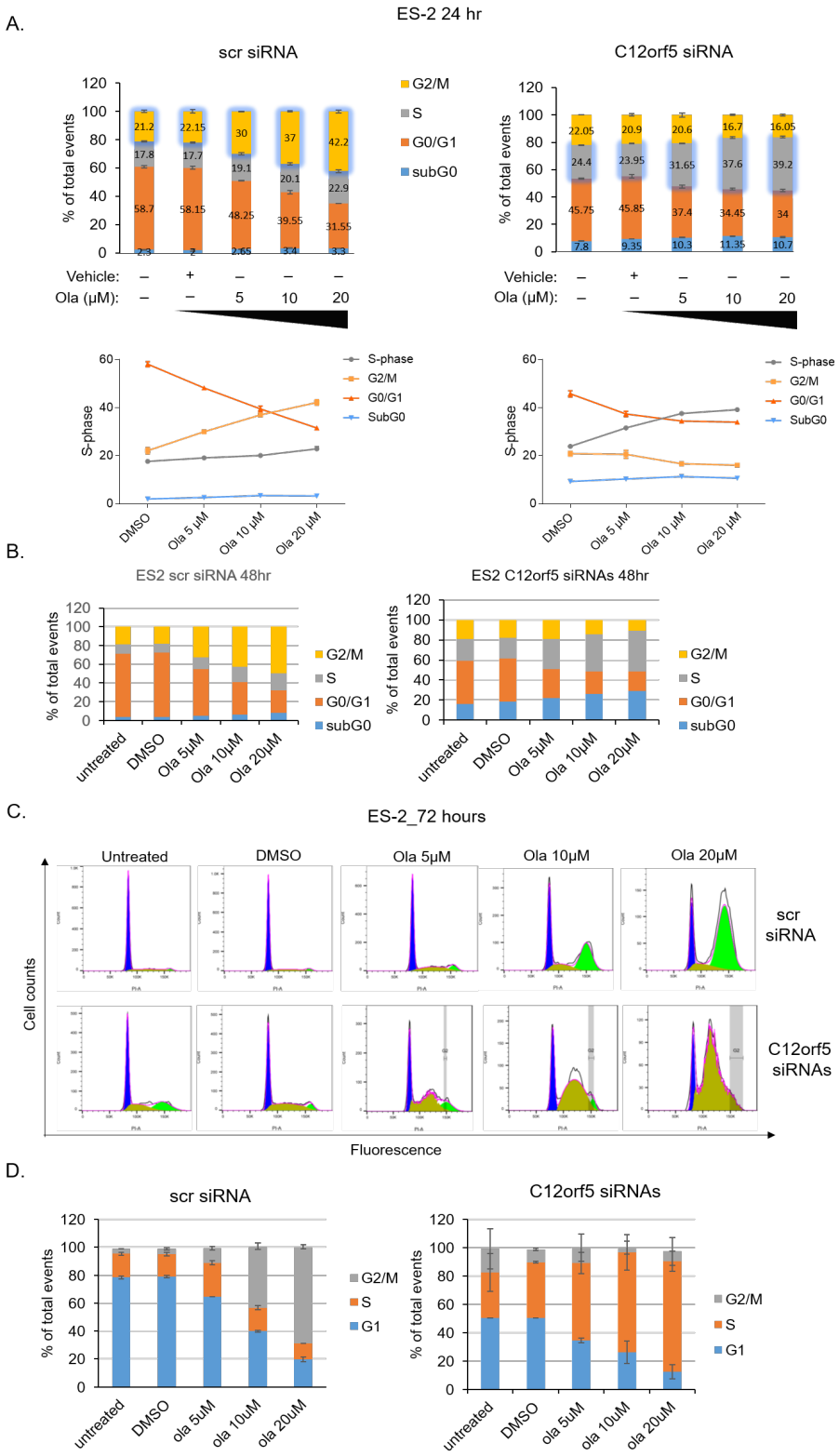

A.
ES-2 24 hr
scr siRNA
C12orf5 siRNA
% of total events
G2/M
S
G0/G1
subG0
Vehicle:
Ola (μM):
S-phase
SubG0
DMSO
Ola 5 μM
Ola 10 μM
Ola 20 μM
B.
ES2 scr siRNA 48hr
ES2 C12orf5 siRNAs 48hr
untreated
DMSO
Ola 5μM
Ola 10μM
Ola 20μM
C.
ES-2_72 hours
Untreated
DMSO
Ola 5μM
Ola 10μM
Ola 20μM
Cell counts
scr siRNA
C12orf5 siRNAs
Fluorescence
D.
scr siRNA
C12orf5 siRNAs
G1
ola 5uM
ola 10uM
ola 20uM

Figure 4.4.6: *TIGAR* knockdown leads to S-phase arrest. A) *TIGAR* knockdown leads to cell cycle arrest in S-phase and the arrest is further enhanced when combined with olaparib treatment. ES-2 cells were transfected with siRNA for 48 hours and treated with vehicle, 5, 10 or 20 μM olaparib for additional 24 hours before flow cytometry cell cycle analysis with PI staining. Data are shown as mean ± SD. **B)** Quantification of cell cycle analysis in ES-2 cells treated with olaparib for 48 hours after siRNA transfection. **C-D)** Cell cycle analysis in ES-2 cells treated with olaparib for 72 hours after siRNA transfection. C) Representative cell cycle profiles analyzed in FlowJo. D) Quantification of cells distributed at different cell cycle phases with 72 hours' of olaparib treatment.

4.4.4 Downregulation of *BRCA1* and the Fanconi anemia (FA) pathway underlies sensitivity to olaparib after TIGAR knockdown

Studies have shown that TIGAR modulates the DDR after treatment with different DNA-damaging agents, such as epirubicin (Yu, Xie et al. 2015) and radiation (Pena-Rico, Calvo-Vidal et al. 2011) and TIGAR downregulation enhances DNA damage with these agents. Together with the data presented in our present study, these effects largely depended on PPP reduction after TIGAR knockdown.

To investigate whether there are other potential mechanism(s) involved in PARP inhibitor sensitivity after TIGAR knockdown, we performed RNA sequencing analysis in OVCA420* cells that were transduced with non-targeting control (NTC) shRNA (short hairpin RNA) or TIGAR-specific shRNA. The efficiencies of three individual TIGAR-specific shRNAs were different in knocking down TIGAR (data not shown), and we found that shRNA #3 gave the most efficient knockdown. Following a 54-hour transduction with lentiviral supernatant, we analyzed changes in the transcriptome using Illumina mRNA sequencing. A total of 380 genes were downregulated by at least two-fold at false discovery rate (FDR) ≤ 0.001 in TIGAR knockdown cells compared to NTC-transduced cells. A total of 144 genes were upregulated by at least two-fold at FDR ≤ 0.001 in TIGAR knockdown cells compared to NTC-transduced cells (Figure 4.4.7.A). Surprisingly, *BRCA1* was one of the most significantly downregulated genes following TIGAR knockdown. Downregulation of *BRCA1* was validated with qRT-PCR and Western blot (Figure 4.4.7.B). Similar results were obtained from another cell line (Figure 4.4.7.F). Interestingly, the extent of *BRCA1* downregulation depended on how efficiently TIGAR was knocked down, suggesting a dose-dependent effect. Gene set enrichment analysis (GSEA) indicated that the TIGAR knockdown cells produced a gene set enrichment similar to *BRCA1* downregulated cells (Figure

4.4.7.C). It is well accepted that downregulation of *BRCA1* leads to PARP inhibitor sensitivity. Our data suggest that TIGAR knockdown induces *"BRCAness"* by phenocopying the gene expression pattern produced by *BRCA1* downregulation, thereby sensitizing cancer cells to olaparib. We also analyzed the differentially expressed genes using Metascape bioinformatics web resources (Tripathi, Pohl et al. 2015). The annotation and enrichment analysis of genes downregulated by TIGAR knockdown indicated that the gene set was enriched for the FA pathway, DNA replication, and cell cycle progression (Figure 4.4.7.D). We validated the downregulation of genes involved in the FA pathway, such as *BRCA2* and *RAD51*, after TIGAR knockdown with two different shRNAs (Figure 4.4.7.E-F). We also validated the decreased expression of downregulated genes with two different shRNAs in two cell lines using qRT-PCR (Figure 4.4.7.G). In general, the extent of the downregulation of these genes was greater when TIGAR was more efficiently downregulated with shRNA. We also performed an analysis for genes upregulated in TIGAR knockdown cells. The results indicated the enrichment of gene ontology for the positive regulation of cell death, response to drug, regulation of PI3K activity, and the positive regulation of intracellular signal transduction (Figure 4.4.7.H). The upregulated genes involved in the positive regulation of cell death is consistent with our observation of an increase in apoptosis after TIGAR knockdown (Figure 4.4.4).

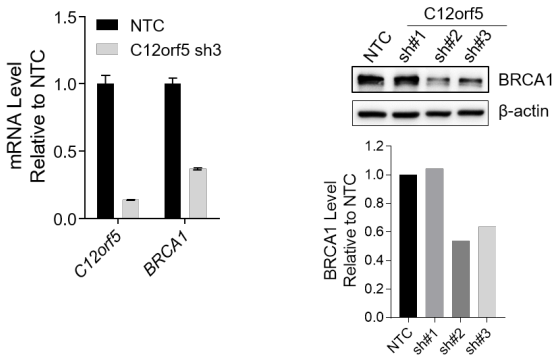
Figure 4.4.7

A.

Significantly changed genes

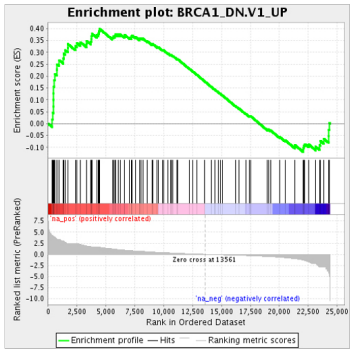
Differentially expressed genes	Number of genes	Fold change	False discovery rate (FDR)
Upregulated in C12orf5 sh#3	144	2-fold	≤0.001
Downregulated in C12orf5 sh#3	380	2-fold	≤0.001

B.



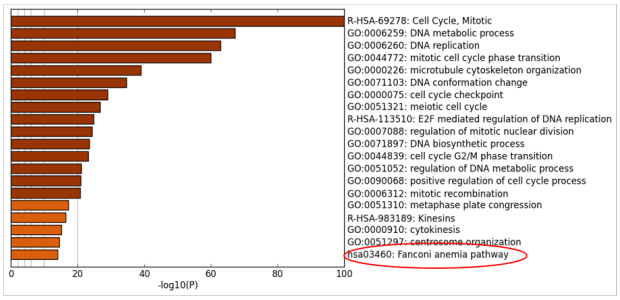
C.

GSEA from RNAseq with C12orf5 sh3

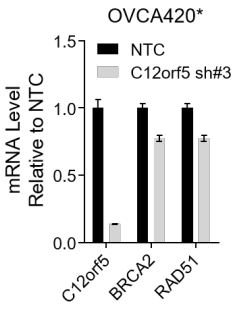


D.

Statistically enriched terms associated with genes downregulated in C12orf5 KD



E.



F.

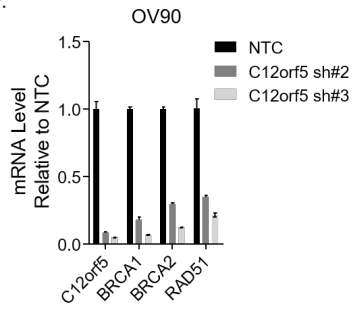
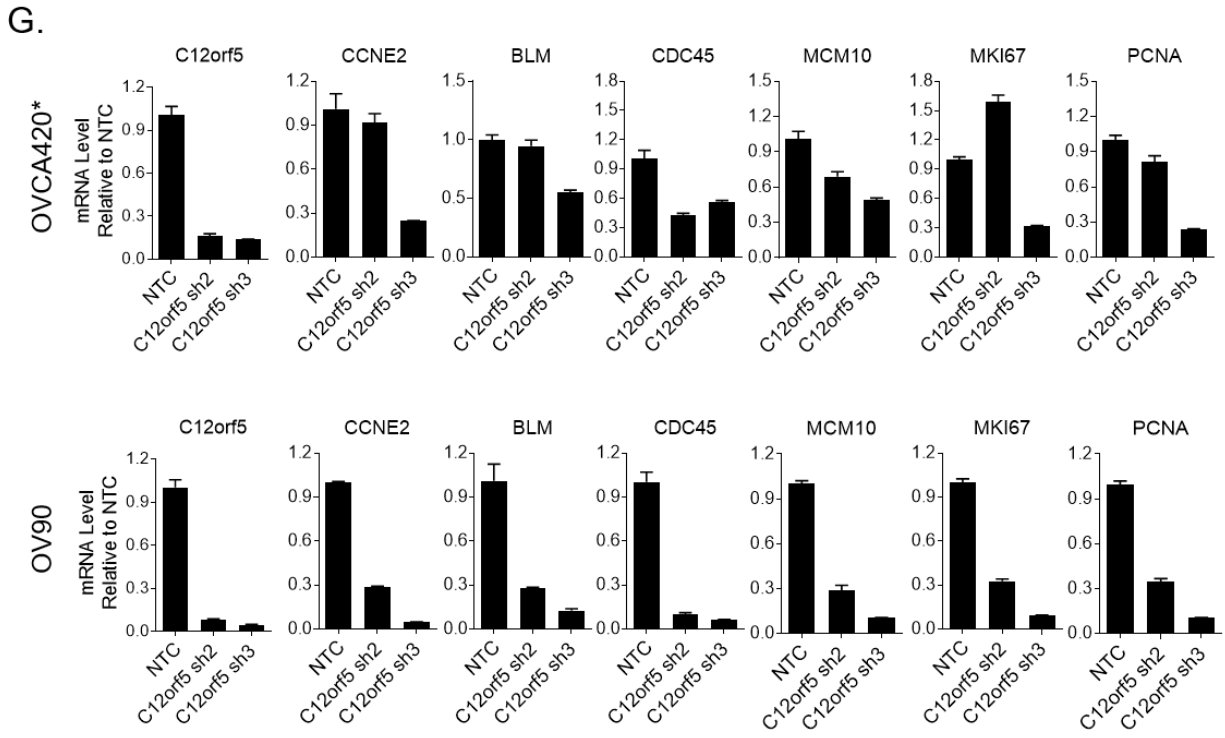


Figure 4.4.7 Cont.



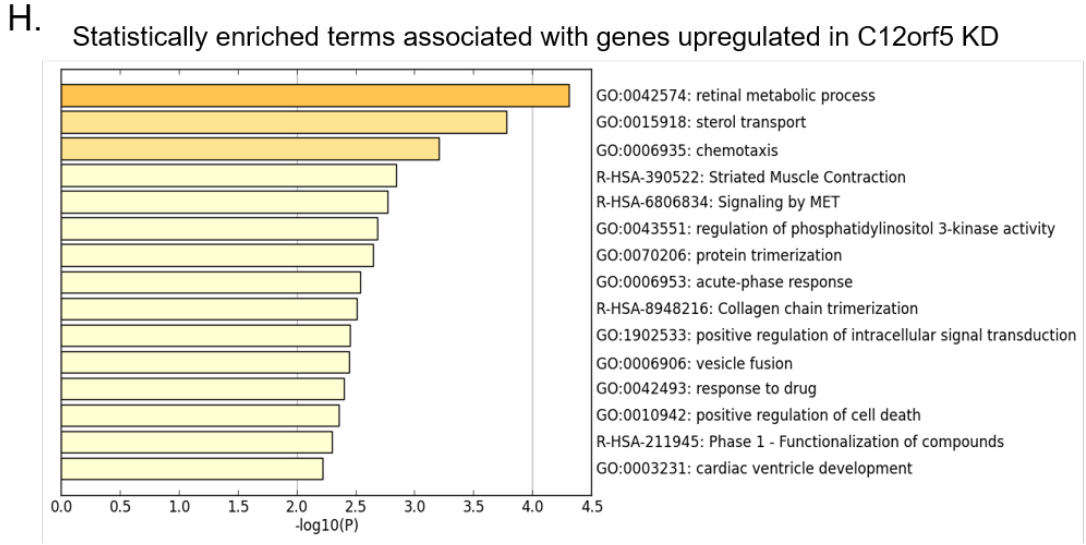


Figure 4.4.7: RNA sequencing analysis after *TIGAR* knockdown reveals downregulation of *BRCA1* and key DNA repair pathways. A) Genes that are significantly changed were identified with Metascape bioinformatics web program. **B)** *BRCA1* is downregulated in OVCA420* cells with *C12orf5* shRNAs. qRT-

PCR and Western blot to check the expression of *BRCA1* mRNA and protein levels after *TIGAR* knockdown with shRNAs. For qRT-PCR, data shown as mean ± SEM. Results represent experiment with triplicates. Representative blot was shown for protein expression check. Bar graph shows densitometry analysis of the western blots. **C)** GSEA analysis reveals enrichment of gene signature that is similar as *BRCA1* downregulated cells after *TIGAR* knockdown. **D)** Statistically enriched terms associated with genes downregulated in *TIGAR* knockdown are related to the Fanconi anemia (FA) pathway, cell cycle progression and etc. **E-F)** The FA pathway components *BRCA1*, *BRCA2*, and *RAD51* were downregulated after *TIGAR* knockdown with shRNAs. QRT-PCR was performed in OVCA420* and OV90 cells at 72 hours after transduction of *TIGAR* shRNA. Data shown as mean ± SEM. Results represent experiment with triplicates. **G)** Validation of expression for downregulated genes in *TIGAR* shRNA transduced OVCA420* and OV90 cells respectively. In general, the extent of downregulation of these genes is bigger when the *TIGAR* is more efficiently downregulated with shRNA. To be specific, *C12orf5* sh#3 downregulate TIGAR more efficient and is accompanies with more downregulation of affected genes. **H)** Statistically enriched terms that are associated with genes upregulated in *TIGAR* knockdown, including positive regulation of cell death, response to drug, regulation of PI3K activity, positive regulation of intracellular signal transduction, etc.

4.4.5 Efficient knockdown of *TIGAR* induces senescence and decreases clonogenicity in cancer cells

Our RNA sequencing data analysis revealed that gene sets involved in DNA replication and cell cycle progression were significantly downregulated. To further characterize the effect of TIGAR knockdown on cancer cell growth, we used three different shRNAs to stably knockdown TIGAR expression in two different cancer cell lines, OVCA420* and OV90. Western blot analysis indicated that three individual shRNAs knocked down TIGAR expression with different efficiencies and were generally more effective in OVCA420* cells (Figure 4.4.8.A). This might be the result of different viral transduction efficiencies between the OVCA420* and OV90 cells. Colony formation assay indicated a marked decrease in clonogenicity following TIGAR knockdown in OVCA420* cells treated with DMSO (Figure 4.4.8.B). Similarly, a decrease in clonogenicity that corresponded with TIGAR knockdown levels was observed in OV90 cells. Clonogenic growth was further decreased by olaparib treatment in OV90 cells.

A previous report by Peña-Rico et al. (2011) showed that TIGAR knockdown with a siRNA in glioblastoma cells induced senescence (Pena-Rico, Calvo-Vidal et al. 2011). To examine if the marked decrease in clonogenicity after TIGAR knockdown with shRNAs is associated with senescence, we performed a senescence-associated β-galactosidase (SA-β-gal) analysis on OVCA420* cells that were transduced with a NTC shRNA or three different shRNAs targeting TIGAR. We consistently observed an increase in SA-β-gal staining in the TIGAR knockdown cells and the extent of cells that underwent senescence was inversely correlated with the level of TIGAR expression (Figure 4.4.8.C-D). These data suggest that more efficient knockdown of TIGAR leads to the induction of senescence and severe cell growth defects in cancer cells, resulting in decreased colony formation. These results suggest that efficient downregulation of

TIGAR by itself may prevent cancer cells from growing, indicating that TIGAR might be a good therapeutic target to explore.

Figure 4.4.8

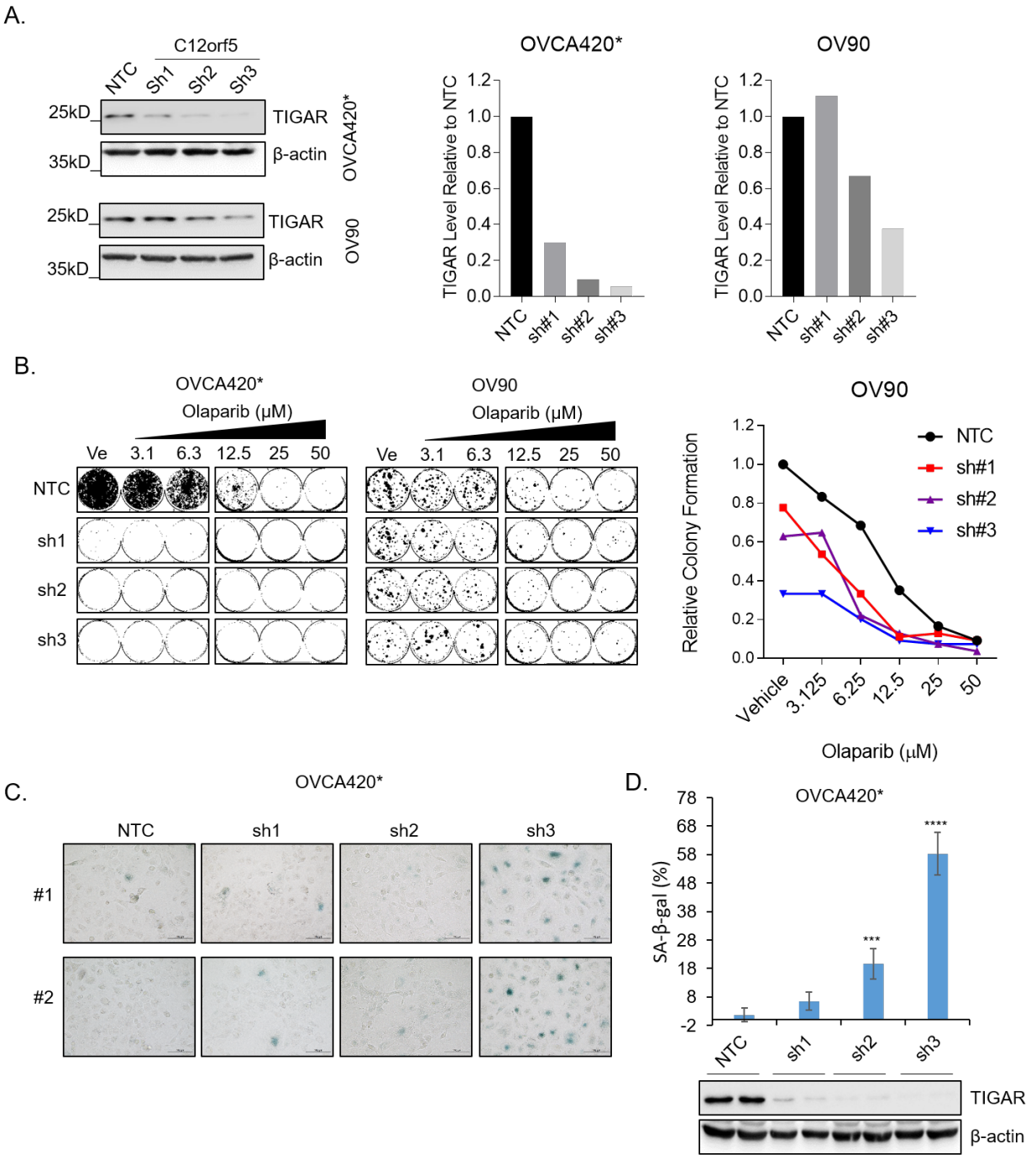


Figure 4.4.8: Efficient *TIGAR* knockdown increases senescence and decreases clonogenicity of cancer cells. (A-B) *TIGAR* knockdown with individual shRNAs decreased colony formation. (A) Western blot to check knockdown efficiency with individual shRNAs. NTC is non-targeting control shRNA. β-actin was

used as a loading control. Bar graphs on the right show the densitometry of western blots in OVCA420* and OV90 cells respectively. (B) Colony formation assay after *TIGAR* shRNA knockdown in OVCA420* and OV90 cells. 72 hours after shRNA viral transduction, cells were seeded for clonogenic assay and treated with vehicle and increasing concentrations of olaparib. Representative images of colonies were shown. Graph on the left showed the quantification of colonies in OV90 cells. **(C-D)** More efficient *TIGAR* knockdown is associated with an increase of senescence in OVCA420* cell. (C) SA-β-gal staining of OVCA420* cells with NTC or individual *TIGAR* shRNAs. Representative images were shown of duplicates from one representative experiment. (D) Quantification of SA-β-gal staining positive cells in each group. The bar graph shows result from one representative experiment with duplicates. Images were taken from different areas in each well for each replicate and combined quantified positive staining cells. Representative western blot showing the corresponding knockdown of *TIGAR* with individual shRNAs. Statistics was done with Student's t-test. $p<=0.05$ was considered to be significant. ***$p<=0.001$, ****$p<=0.0001$.

4.4.6 *TIGAR* knockdown enhances therapeutic effects of olaparib in cancer cells

Three-dimensional (3D) cell culture models are used as intermediate models between *in vitro* two-dimensional (2D) monolayer cancer cell culture and *in vivo* tumors (Weiswald, Bellet et al. 2015). Emerging evidence suggests that 3D culture systems represent more accurately the actual tumor microenvironment than 2D culture systems (Edmondson, Broglie et al. 2014). Further, 3D culture models are considered more representative than 2D systems in testing the therapeutic effects of drugs *in vitro* (Edmondson, Broglie et al. 2014, Weiswald, Bellet et al. 2015, Zanoni, Piccinini et al. 2016). To test the therapeutic effects of the combination of olaparib with TIGAR downregulation, we decided to use inducible shRNAs to perform the 3D spheroids formation assay. With two different inducible shRNAs against TIGAR, we observed a significant decrease in cell viability in spheroids and sizes of spheroids when olaparib treatment was combined with TIGAR knockdown (Figure 4.4.9.A-C). These data indicate that TIGAR knockdown enhances the therapeutic effects of olaparib, which is consistent with our findings in RNA sequencing analysis that *BRCA1* and the FA pathway are significantly downregulated after TIGAR knockdown.

Figure 4.4.9

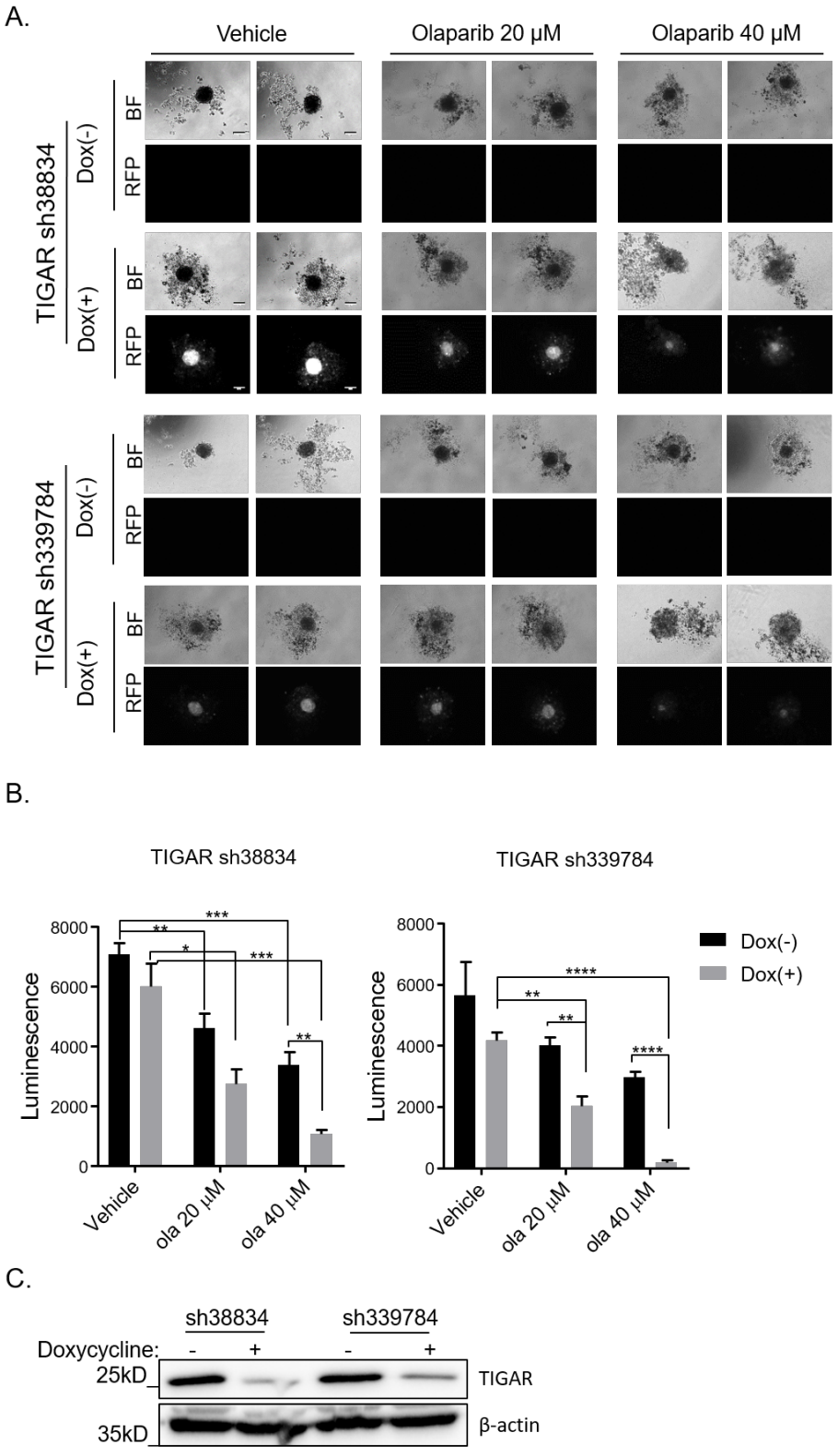

A.
Vehicle
Olaparib 20 μM
Olaparib 40 μM
TIGAR sh38834
TIGAR sh339784
Dox(-)
Dox(+)
BF
RFP
B.
TIGAR sh38834
TIGAR sh339784
Luminescence
Dox(-)
Dox(+)
Vehicle
ola 20 μM
ola 40 μM
C.
sh38834
sh339784
Doxycycline:
25kD
35kD
TIGAR
β-actin

Figure 4.4.9: *TIGAR* knockdown enhances therapeutic effects of olaparib. A-C) 3D spheroid formation assay indicates enhanced therapeutic effects of olaparib combined with *TIGAR* knockdown using two different inducible shRNAs. (**A**) Representative images of spheroids from each group. Two different spheroids were shown from each group. OVCA420* cells stably carrying inducible shRNAs were induced with 1 μg/mL doxycycline for 72 hours before seeded into 1.5% agarose coated 96 well plate with 3000 cells per well. Cells were treated with vehicle, 20 μM or 40 μM olaparib for 10 days after spheroids formed. 1 μg/mL of doxycycline was kept in the media throughout the whole experiment. Expression of RFP (Red Fluorescent Protein) is an indication of shRNA expression. BF, bright field. (**B**) Cell viability of spheroids was determined with CellTiter-Glo Luminescent Cell Viability Assay. Data are shown as mean ± SEM. Statistics was done with Student's t-test. p<=0.05 was considered to be significant. *p<=0.05, **p<=0.01, ***p<=0.001, ****p<=0.0001. (**C**) Western blot to show that *TIGAR* was knocked down with doxycycline induction. Cells induced with doxycycline were collected for western blot in parallel of the spheroids formation assay.

4.4.7 *TIGAR* expression in ovarian carcinomas is associated with poor survival outcome

Finally, data analysis from TCGA indicated that TIGAR was amplified across different cancer types. This includes ovarian cancer, which was amplified in over 11% of cases (Figure 4.4.10.A). Further, TIGAR amplification was correlated with a higher expression of TIGAR (Figure 4.4.10.B). We also analyzed the RNA sequencing dataset of high-grade serous ovarian carcinomas from TCGA. We classified patients into two groups depending on the levels of TIGAR expression in the tumor. Tumors with TIGAR expression above the mean were considered tumors with a high expression of TIGAR whereas tumors with TIGAR expression at or below the mean were considered tumors with a low expression of TIGAR. The analysis indicated that patients with high TIGAR expression in tumors had a significantly poorer overall survival outcome compared to patients with low TIGAR expression in tumors (Figure 4.4.10.C). Collectively, these results suggest an important role of TIGAR in cancer development and in modifying the response to the PARP inhibitor. Finally, targeting TIGAR in cancer represents a potential strategy to overcome PARP inhibitor resistance and prevent tumor progression.

Figure 4.4.10

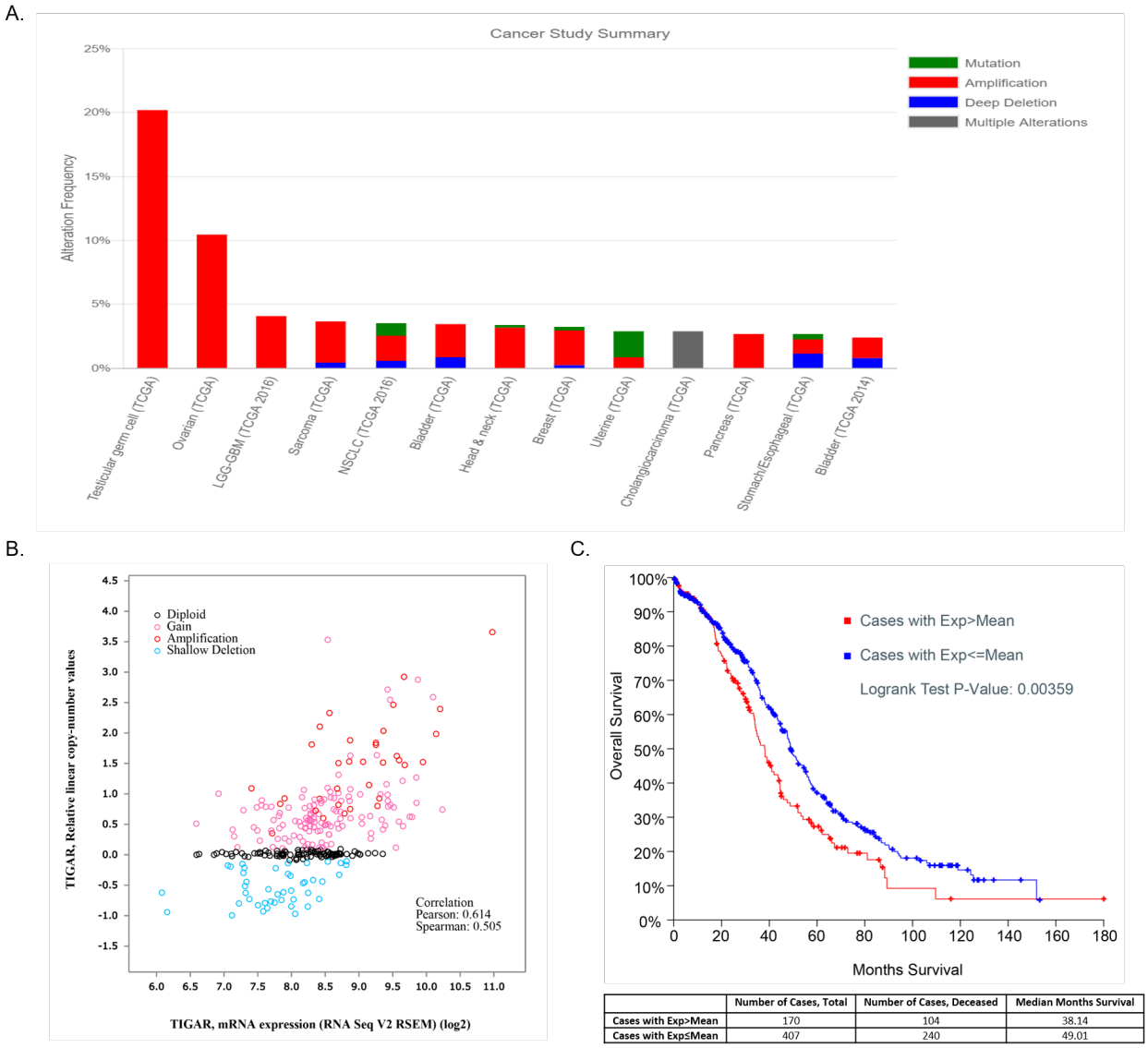


Figure 4.4.10: Clinical relevance of therapeutically targeting TIGAR in ovarian cancer. A) *TIGAR* is amplified in ~10% of serous ovarian tumor samples analyzed through The Cancer Genome Atlas (TCGA). These levels are derived from the copy-number analysis algorithms GISTIC or RAE, and indicate the copy-number level per gene. "-2" is a deep loss, possibly a homozygous deletion, "-1" is a shallow loss (possibly heterozygous deletion), "0" is diploid, "1" indicates a low-level gain, and "2" is a high-level amplification.

And these calls are putative. **B)** TIGAR amplification was correlated with a higher expression. (**C)** Higher *TIGAR* expression is associated with poor survival in high-grade serous ovarian cancer patients. 489 patients from TCGA publication was included in the analysis. mRNA expression was quantified by Agilent microarray. *TIGAR* expression ≥1.5 standard deviations above the mean is considered overexpressed.

4.5 Discussion

Several mechanisms have been proposed for PARP inhibitor resistance, such as alterations that directly or indirectly restore HR function, including the reversion mutation of BRCA1/2, epigenetic re-expression of BRCA1, and overexpression of 53BP1 and stabilization of partially truncated BRCA1 (Lord and Ashworth 2013, Ter Brugge, Kristel et al. 2016, Incorvaia, Passiglia et al. 2017). Other non-HR mechanisms, such as the loss of PARP1 expression, PI3K/AKT pathway, and an increase in the drug transporter P-glycoprotein to facilitate the efflux of drugs also contribute to PARP inhibitor resistance (Tapodi, Debreceni et al. 2005, Liu, Han et al. 2009, Pettitt, Rehman et al. 2013, Lawlor, Martin et al. 2014, Durmus, Sparidans et al. 2015). However, our knowledge about PARP inhibitor resistance is far from complete and more effective combination therapies need to be developed to overcome the resistance. RNAi-based functional genetic screens are valuable techniques to efficiently identify a set of genes underlying a particular cellular process (Fire, Xu et al. 1998, Brummelkamp, Bernards et al. 2002, Moffat, Grueneberg et al. 2006, Ngo, Davis et al. 2006). Although they are powerful tools to disrupt gene function, they suffer from the low suppression of gene levels and frequent off-target effects, resulting in high rates of false positive and negative results (Echeverri, Beachy et al. 2006, Booker, Samsonova et al. 2011, Kaelin 2012). The CRISPR/Cas9-based system has been shown as advantageous in these aspects and pooled gRNA screens as effective in loss-of-function screens in mammalian cells as well as in vivo screens in mice (Shalem, Sanjana et al. 2014, Wang, Wei et al. 2014, Chen, Sanjana et al. 2015, Parnas, Jovanovic et al. 2015). Several published studies reported siRNA or shRNA library screens to characterize modifiers of the PARP inhibitor response (Lord, McDonald et al. 2008, Bajrami, Frankum et al. 2014). Although our report is not the first to perform the

CRISPR/Cas9 screen to identify modifiers of the PARP inhibitor response (Pettitt, Krastev et al. 2017), it is the first to report TIGAR as a candidate modifier of the response to olaparib.

TIGAR was originally described as a p53-induced gene that regulates glycolysis and apoptosis (Bensaad, Tsuruta et al. 2006). However, subsequent studies described its additional role in DNA damage signaling, mitophagy, autophagy, and senescence (Bensaad, Tsuruta et al. 2006, Bensaad, Cheung et al. 2009, Yu, Xie et al. 2015). In cancer cells, TIGAR plays an important role in regulating glycolytic flux and promotes NADPH and antioxidant regeneration through oxidative PPP (Lee, Vousden et al. 2014). TIGAR knockdown results in a decrease in NADPH, an increase in ROS, and higher basal levels of DNA damage (Bensaad, Tsuruta et al. 2006, Yu, Xie et al. 2015). A previous report indicated that TIGAR knockdown results in the senescence of glioblastoma cells (Pena-Rico, Calvo-Vidal et al. 2011). Given its pivotal role in antioxidant regeneration, it is not surprising that our genome-scale CRISPR/Cas9 screen identified *C12orf5* (TIGAR) as a dropout candidate gene involved in sensitizing cancer cells to PARP inhibitor olaparib.

Our screen indicated that all three specific gRNAs for TIGAR were significantly depleted following exposure to 5 or 10 μM olaparib (Figure 4.4.2). Our studies showed that TIGAR-deficient cells were more sensitive to olaparib (Figure 4.4.3). TIGAR knockdown by RNAi resulted in increased levels of ROS and higher basal levels of DNA damage (Figure 4.4.5). Consistent with previous studies (Farmer, McCabe et al. 2005), the cancer cells in our study show an increased accumulation in the G_2 or M phase when they were treated with olaparib. In contrast, TIGAR-deficient cells showed an increased accumulation in the S-phase when they were treated with olaparib (Figure 4.4.6). These results are consistent with our observation that TIGAR-deficient cells sustained more DNA damage, thereby requiring a longer time to resolve DNA

damage in the S-phase. TIGAR knockdown also led to growth inhibition and is supported by our observations of an increase in apoptosis (Figure 4.4.4) and induction of senescence (Figure 4.4.8). These results correlate with the analysis of the global changes in the transcriptome following TIGAR knockdown that showed that genes involved in cell cycle progression were most significantly affected by TIGAR knockdown (Figure 4.4.7). More interestingly, TIGAR knockdown led to downregulation of *BRCA1* (Figure 4.4.7). GSEA from RNA sequencing indicated that TIGAR knockdown produced a gene signature similar to *BRCA1* downregulated cancer cells (Figure 4.4.7). Moreover, pathway analysis showed that one of the significant gene ontology (GO) terms associated with downregulated genes is the FA pathway (Figure 4.4.7). BRCA1 is a tumor suppressor that is involved in a handful of important cellular processes and its role in HR and transcriptional regulation in DDRs draws the most attention (Yoshida and Miki 2004, Silver and Livingston 2012). BRCA1 deficiency leads to HR defects and the sensitization of cancer cells to the PARP inhibitor (Farmer, McCabe et al. 2005). The FA pathway regulates DNA repair by HR and defects in the FA pathway lead to DNA repair defects, resulting in a low tolerance for DNA-damaging agents (Kennedy and D'Andrea 2005). Accordingly, downregulation of BRCA1 and the FA pathway in TIGAR-deficient cells may provide a molecular mechanism attributable to the increased sensitivity to olaparib after TIGAR knockdown in cancer cells. Targeting TIGAR may induce *"BRCAness"* in cancer cells and serve as a combination strategy to overcome PARP inhibitor resistance or to enhance the therapeutic effects of the PARP inhibitor. Our data from spheroids formation assay suggest that downregulation of TIGAR enhanced the therapeutic effects of olaparib (Figure 4.4.9). In the future, it will be informative to test the effects of TIGAR downregulation in combination with the PARP inhibitor *in vivo.* Importantly, TCGA data analysis indicated that a higher expression of TIGAR was observed in many different cancer

types and patients with a higher TIGAR expression showed poor overall survival outcome in high-grade serous ovarian cancer (Figure 4.4.10). These data suggest that the clinical relevance of TIGAR expression in cancer and the targeting of TIGAR might be a useful strategy to improve cancer treatments.

Along with our present study, several studies have shown the potential of targeting TIGAR to enhance the therapeutic effects of cancer therapies. These include the direct targeting of TIGAR with a siRNA (Pena-Rico, Calvo-Vidal et al. 2011) or miR-144 (Chen, Li et al. 2015) and indirect downregulation with a cell-penetrating peptide inhibitor of MUC1-C (Yin, Kufe et al. 2014, Ahmad, Alam et al. 2017) or c-Met TKIs (Lui, Wong et al. 2011). Therefore, the future development of pharmacological methods to directly target TIGAR is an attractive direction to pursue. TIGAR is characterized as an F-2,6-BPase that could be targeted by specific phosphatase inhibitors to inhibit its activity and inhibit the PPP to enhance the cytotoxicity of cancer therapies as suggested by our data and reports from other groups (Pena-Rico, Calvo-Vidal et al. 2011, Yin, Kufe et al. 2014). Degraders based on proteolysis targeting chimera that specifically target TIGAR can also be developed.

One potentially promising aspect of targeting TIGAR in cancer is that it regulates the Warburg effect. This effect acts as a metabolic switch that enables cancer cells to utilize higher amounts of glucose by shunting glucose-6-phosphate to the PPP, which is in part mediated by TIGAR. This metabolic feature is almost universal in cancer. Therefore, it may represent a critical step in cancer progression. The beneficial effect of this metabolic feature for cancer cells is that it provides critical substrates for DNA and, thus, enables replication. Further, the feature provides critical substrates for the regeneration of antioxidants, thereby protecting cancer cells from excessive oxidative damage through accelerated cellular metabolism. Therefore, targeting TIGAR

may not only deplete the cancer cells with critical substrates required for proliferation but also deprive them of the substrates required for antioxidant regeneration. The combined effect of TIGAR inhibition results in increased ROS, DNA damage, cellular senescence, and increased sensitivity to PARP inhibitors.

Chapter 5. Overall discussion and future directions

Although considered a rare cancer (representing only 1.3% of all new cancer cases in the U.S), ovarian cancer is the deadliest gynecological cancer among women in the US (American Cancer Society 2017). Due to the lack of reliable early detection biomarkers (Einhorn, Sjovall et al. 1992, DePriest and DeSimone 2003, Wilder, Pavlik et al. 2003, Jacobs and Menon 2004, Olivier, Lubsen-Brandsma et al. 2006), the majority of ovarian cancer patients are diagnosed at advanced stages (stage III or IV) where the disease has spread outside the pelvis. Because accurate early detection methods are not yet available and are challenging to develop (Visintin, Feng et al. 2008), effective cancer treatments become more important to improve the outcome of patients with ovarian cancer. The standard chemotherapy, consisting of a platinum and a taxane agent, is considered the standard of care for advanced ovarian cancer patients following surgical debulking (Cannistra 2004). Though these chemotherapies are effective, they are not durable (Cannistra 2004, Chien, Kuang et al. 2013). Efforts to uncover oncogenic driver mutations that can be targeted with molecularly targeted therapies were unsuccessful because high-grade serous ovarian cancer commonly harbor mutations in tumor suppressor genes than mutations in oncogenes (Cancer Genome Atlas Research 2011). However, genomics studies uncovered HR-deficiencies in approximately 50% of high-grade serous ovarian cancer. Recent advances in targeted therapies that exploit synthetic lethal genetic interactions indicate that HR-deficiencies in cancer can be exploited with PARP inhibitors. Two fundamental landmark studies showed that BRCA-deficient cancer cells are hypersensitive to PARP inhibitor (Bryant, Schultz et al. 2005, Farmer, McCabe et al. 2005). These studies extended the historically "old" concept of synthetic lethality to cancer treatment and for the first time provided a new approach to target the loss-of-function mutations in tumor suppressor genes. Since then, PARP inhibitors have been brought into clinical trials (Fong, Boss et al. 2009, Audeh, Carmichael et al. 2010, Tutt, Robson et al. 2010, Ledermann,

Harter et al. 2012, Ledermann, Harter et al. 2014, Mirza, Monk et al. 2016), and based on their promising activities in cancer patients, they were finally approved for treating ovarian cancer patients with deleterious germline or somatic *BRCA* mutations. PARP inhibitors are now evaluated in different phases of clinical trials in various types of cancers, with the hope of expanding their clinical applications. It is now well documented that clinical activities of PARP inhibitors extend beyond BRCA deficiencies, and tumors with HR deficiencies are also sensitive to PARP inhibitors.

In both preclinical and clinical studies, PARP inhibitors are shown to be more effective in cancer cells with HR deficiency and in patients with the platinum-sensitive disease (Bryant, Schultz et al. 2005, Farmer, McCabe et al. 2005, Fong, Boss et al. 2009, Audeh, Carmichael et al. 2010, Tutt, Robson et al. 2010, Lord and Ashworth 2013, Kristeleit, Shapira-Frommer et al. 2016, Coleman, Oza et al. 2017). For example, results from a clinical trial with niraparib showed a clear benefit in the BRCA-deficient group and the platinum-sensitive group (Oza, Tinker et al. 2017), with no response in platinum-resistant BRCA-proficient patients. HR deficiency was shown to confer sensitivity to platinum agents (Yang, Khan et al. 2011, Bolton, Chenevix-Trench et al. 2012, Pennington, Walsh et al. 2014, Konstantinopoulos, Ceccaldi et al. 2015), suggesting that functional status of HR determines the responsiveness of cancer cells to PARP inhibitor and platinum-based chemotherapy. Hence, the intrinsic PARP inhibitor resistance can be explained by the lack of HR defects in cancer cells while the acquired PARP inhibitor resistance is attributed to the restoration of HR proficiency. Therefore, overcoming the HR proficiency is a major challenge in expanding the clinical application and maintaining clinical benefits of PARP inhibitors in cancer treatment. Given that only half of the patients with high-grade serous carcinomas have HRD tumors, current application of PARP inhibitors as a treatment for ovarian cancer will be limited. For the other half, some patients have tumors that maintain HR proficiency due to *CCNE1* amplification, and they

are refractory to platinum agents and expected to be insensitive to PARP inhibitors. In these patients with *CCNE1* amplification, the induction of a *"BRCAness"* phenotype may represent a therapeutic approach because CCNE1 amplification and BRCA deficiency are mutually exclusive, suggest a potential synthetic lethality. Our results indicate that targeting FOXM1-MYC nexus with BET inhibitor or BET degrader induce *"BRCAness"* by causing the downregulation of HR repair genes. Therefore, these agents may be active in treating patients with CCNE1 amplifications who are refractory to platinum-based chemotherapy.

The HR status is unknown for the remaining group that does not have *CCNE* amplification nor known defects in HR genes. For this group of patients, there is no indication that PARP inhibitors will be effective, and this group lacks known predictive signatures of HR defects or HR proficiency (Konstantinopoulos, Ceccaldi et al. 2015). To address this gap in knowledge, more efforts are needed to identify determinants and modifiers of the HR function and PARP inhibitor response. More studies are also needed to explore strategies to induce HR deficiency or *"BRCAness"* in cancer cells with primary resistance to PARP inhibitors so that more patients will benefit from PARP inhibitor treatment. One approach is to combine therapies that impair HR proficiency with PARP inhibitors and make cancer cells sensitive to PARP inhibitors. However, this approach has serious limitations that have to be overcome before it can become a valid clinical option. The primary limitation is that such approach may also induce *"BRCAness"* in normal cells, thereby increasing the risk of adverse side effects. However, novel approaches can be envisioned that selectively induce *"BRCAness"* in cancer cells through exploiting vulnerabilities in protein quality control system in cancer cells. Due to copy number alterations and mutations that exacerbate proteotoxic stress in cancer cells, inhibition of protein chaperones and proteasomal degradation may have more pronounced effects in cancer cells than in normal cells. Prior studies

have shown that HSP90 inhibitors and proteasomal inhibitors induce *"BRCAness"* and sensitize cancer cells to PARP inhibitors. Therefore, it may be possible to selectively cause severe proteotoxic stress and induce *"BRCAness"* while causing minimal effects in normal cells. Under this condition, PARP inhibitors may be more active in cancer cells than in normal cells.

In this dissertation, I focused on two main parts. In Chapters 2 and 3, I described the development of new combination strategies to induce *"BRCAness"* in HR-proficient cells so that these cells become sensitive to PARP inhibitor. I demonstrated that *"BRCAness"* can be induced in cancer cells with either the intrinsic resistance or the acquired resistance to PARP inhibitors and induced *"BRCAness"* in these cells enhances sensitivity to PARP inhibitors. In Chapter 4, I described the characterization of TIGAR as novel modifier of PARP inhibitor response, and this discovery may provide us with additional predictive biomarkers to evaluate the functional status of HR and better predict the PARP inhibitor response. It can also provide us a new potential therapeutic target to explore for development of novel combinational therapies to enhance PARP inhibitor effects.

In Chapters 2 and 3, I focused on inducing *"BRCAness"* by targeting FOXM1 pathway. FOXM1 is an oncogenic transcription factor that has been implicated in many cellular processes (Lam, Brosens et al. 2013, Konstantinopoulos, Ceccaldi et al. 2015). Emerging evidence suggests that FOXM1 can be considered as a master regulator of DNA damage response, oxidative stress response, and resistance to genotoxic agents (Zona, Bella et al. 2014), because FOXM1 transactivates genes involved in DNA damage response, oxidative stress response, and DNA repair, leading to enhanced DNA repair and resistance to DNA damaging agents (Raychaudhuri and Park 2011). In high-grade serous ovarian cancer, FOXM1 pathway is activated in more than 84% of cases (Cancer Genome Atlas Research 2011), thus making it a valid therapeutic target to

explore. In Chapter 2, I showed a new role of FOXM1 in the adaptive response to PARP inhibitor olaparib in cancer cells. I showed that olaparib induces FOXM1 expression which in turn induces the expression of DNA repair genes, thus constitution an adaptive response to olaparib-induced DNA damages. Upon induction by olaparib, FOXM1 translocates to the nucleus, binds to the promoters of genes involved in DNA repair, and upregulates their expression. Knockdown or chemical inhibition of FOXM1 sensitizes cancer cells to PARP inhibitor. Thiostrepton, as a FOXM1 inhibitor, increases the sensitivity of cells to PARP inhibitor. We showed that thiostrepton treatment leads to inhibition of FOXM1 and downregulation of HR genes, thereby inducing *"BRCAness"* and sensitizing resistant cells to PARP inhibitors. Meanwhile, we observed that DNA damage is enhanced when thiostrepton was combined with PARP inhibitor, and this result is consistent with the downregulation of HR genes, which would result in decreased DNA repair. The decrease of HR genes after FOXM1 inhibition with thiostrepton might partially explain the increase of DNA damage that we observed. Thiostrepton has been shown to induce intracellular ROS levels (Bowling, Doudican et al. 2008), which might also contribute to the increase of DNA damage. FOXM1 regulates anti-oxidant genes involved in scavenging ROS, and therefore inhibition of FOXM1 by thiostrepton may attenuate the expression of anti-oxidant genes and exacerbate ROS levels. Besides being a FOXM1 inhibitor, thiostrepton was also shown to be a proteasome inhibitor (Aminake, Schoof et al. 2011, Gartel 2011). Proteasome inhibition can induce cellular apoptosis (Concannon, Koehler et al. 2007). Our observation of increased cellular apoptosis after thiostrepton treatment might partially due to its proteasome inhibitor activity. Collectively, these results suggest that the sensitization of resistant cancer cells to PARP inhibitor by thiostrepton can only be partially explained by inhibition of FOXM1. In this aspect, more specific FOXM1 inhibitors may be more advantageous and desirable. Two additional FOXM1

inhibitors siomycin A (Gartel 2013) and FDI-6 (Gormally, Dexheimer et al. 2014) are now available, and FDI-6 has been shown to be a more specific FOXM1 inhibitor compared to thiostrepton and siomycin. However, FDI-6 was soluble in DMSO, and high concentrations are needed to suppress FOXM1, which might present a challenge for *in vivo* study. It is questionable whether the effect of FDI-6 can be obtained at the clinically achievable doses. In the future, the focus should be put on the development of more specific and more drug-like FOXM1 inhibitors.

In Chapter 3, we first evaluated the extent to which *FOXM1* knockdown affects the expression of HR genes. Surprisingly, the mRNA levels of *FOXM1* target genes in HR pathway, such as *BRCA1, BRCA2, BRIP1* and *FANCF* were maintained after *FOXM1* knockdown by siRNAs. Interestingly, c-MYC was seen to be upregulated in these *FOXM1* knockdown cells. So, we tested the hypothesis of HR genes may be co-regulated by both FOXM1 and c-MYC. Our hypothesis was supported by data from ENCODE, which indicates overlapping binding sites for both FOXM1 and c-MYC in the regulatory regions of target genes. TCGA data analysis reveals that expression of *FOXM1* and *MYC* are elevated in ovarian cancer, and their expressions are negatively correlated, further supporting our *in vitro* finding that *FOXM1* knockdown upregulates c-MYC expression. Given that BRD4 regulates the expression of both FOXM1 and MYC, we also analyzed the expression of BRD4 in TCGA datasets, and our analysis shows that *BRD4* expression is the highest in ovarian carcinoma samples compared to 23 other cancer types. Consistent with the literature that BRD4 regulates the expression of FOXM1 and MYC, we observed a positive correlation in gene expression between BRD4 and FOXM1 or MYC. Given that BRD4 is highly expressed in ovarian cancer and that it positively regulates FOXM1 and MYC, we posit that BRD4 inhibition will suppress the expression of FOXM1 and MYC and attenuate the expression of DNA repair genes, thereby inducing *"BRCAness"*. We also posit that the ensuing *"BRCAness"* will

enhance sensitivity to PARP inhibitors. Given that BRD4 inhibitor and degrader are available as experimental therapeutics (Filippakopoulos, Qi et al. 2010), we tested their effects on FOXM1 and c-MYC expression in ovarian cancer cells. We observed that BRD4 inhibitor (+)-JQ1 suppresses the expression of FOXM1 and c-MYC and efficiently downregulates of HR genes, such as *BRCA1* and *RAD51*, suggesting BET inhibitor is effective in induction of *"BRCAness"*. We observed similar effects with BET degrader ZBC260. With BET inhibitor (+)-JQ1, we demonstrated increased sensitivity to PARP inhibitors in HR-proficient PARP inhibitor resistant cancer cells. Our ChIP data indicates a decrease binding of FOXM1 and c-MYC to the promoters of *BRCA1* and *RAD51* following (+)-JQ1 treatment, which may provide a molecular mechanism by which (+)-JQ1 downregulates the expression of *BRCA1* and *RAD51*. These results also support our initial analysis of ENCODE data indicating that these genes may be co-regulated by both FOXM1 and c-MYC. Our study provided new insight into the regulation of DNA repair genes especially HR genes by c-MYC and the interplay between FOXM1 and c-MYC in this process. The FOXM1 pathway was shown to be downregulated by BET inhibitors (Zhang, Ma et al. 2016), which was suggested to be a therapeutic strategy in ovarian cancer. Our data provide additional evidence to support this by showing downregulation of HR genes resulted from FOXM1 inhibition by BET inhibitors. The observation that combinations of sub-lethal doses of BET inhibitor and PARP inhibitor completely inhibited colony formation provides a new basis for the further clinical development of combination therapies targeting these two components. Lu et al., has tested the potential of BET inhibitor to enhance effects of olaparib in HR-proficient tumor xenografts from primary resistant cancer cells, however, the *in vivo* activity of this combination has not been shown in the acquired resistant cancer cells, and the potential has not yet been tested in *MYC*-amplified tumor models neither. In addition, we tested a more potent PROTAC-based BET inhibitor,

ZBC260, which has been shown active in *in vivo* models (Zhou, Hu et al. 2017). Future studies are needed to evaluate the *in vivo* combined effects of ZBC260 and PARP inhibitor.

In addition, there are other important questions that need to be addressed. First, it will be important to understand how FOXM1 is downregulated following exposure of cells to BET inhibitors, which might involve the mechanisms of how BRD4 regulates FOXM1 expression. Second, it is possible that downregulation of *BRCA1* and *RAD51* is independent of FOXM1 and is the direct effect of BRD4 inhibition. A newly published study showed that transcription of *BRCA1* and *RAD51* was impaired by the depletion of BET proteins, and BET inhibitor (+)-JQ1 directly repressed the promoter activities of *BRCA1* and *RAD51* (Yang, Zhang et al. 2017). However, the contribution of FOXM1 in JQ1-mediated downregulation of BRCA1 and RAD51 was not addressed in the study. Given that BET proteins are chromatin readers and serve as co-transcription factors, these proteins may bind to the regulatory regions and recruit additional transcription factors, such as FOXM1 and c-MYC for transactivation of target genes. Taken together, it is possible that downregulation of *BRCA1* and *RAD51* by BET inhibitor might be the result of a combined effects of inhibition of FOXM1, c-MYC, and BET proteins, such as BRD2, BRD3, and BRD4. Third, in ES-2 cells, we did not observe efficient downregulation of c-MYC by BET inhibitors, suggesting that *MYC* expression in this cell line is not primarily dependent on BET proteins. It would be interesting to know what factors regulate *MYC* expression in these cells. Interestingly, we detected efficient downregulation of *BRCA1* and *RAD51* in these cells by the BET inhibitor, which suggest *BRCA1* and *RAD51* are directly regulated by BET proteins or MYC-independent transcription factors. The role of FOXM1 in transactivation of BRCA1 and RAD51 cannot be ruled out because (+)-JQ1 consistently downregulates FOXM1 expression in cancer cells. Fourth, it will be informative to determine changes in the expression of DNA repair genes

regulated by FOXM1 and c-MYC after deletion of either one or both of them. We previously used siRNAs to knockdown FOXM1, c-MYC, or both FOXM1 & c-MYC, but we did not observe downregulation of candidate DNA repair genes even in a double knockdown (data not shown). However, we were unable to come to a conclusion that FOXM1 and c-MYC do not regulate the expression of these genes because the gene expression was performed at 72 hours after siRNA transfection, and other transcription factors could have upregulated the expression of these genes as a compensation mechanism to accommodate the downregulation of FOXM1 and c-MYC. A transient knockdown of FOXM1 and MYC by inducible shRNAs may be needed to resolve this issue.

In order to achieve a better understanding of the regulation of HR pathway and to identify novel modifiers of PARP inhibitor response in ovarian cancer cells, in Chapter 4, I described the CRISPR/Cas9-based knockout genetic screen that we performed. Using the sensitivity to the FDA-approved PARP inhibitor olaparib as a readout, we identified top candidates that are contributing to PARP inhibitor sensitivity and resistance, respectively. For example, *PARP1* is one of the sensitivity candidates, loss of which leads to PARP inhibitor resistance. This finding is consistent with the previous report that loss of PARP1 leading to PARP inhibitor resistance (Liu, Han et al. 2009, Pettitt, Rehman et al. 2013), and the fact that *PARP1* was picked up from the screen suggests that our screen worked. In addition, we identified *C12orf5* (*TIGAR*) as one of the candidate genes that confer PARP inhibitor resistance. *C12orf5* gene encodes TIGAR (*TP53*-Induced Glycolysis and Apoptosis Regulator), which is described as a fructose-2, 6-bisphosphatase (Bensaad, Tsuruta et al. 2006). TIGAR functions primarily as a regulator of glucose breakdown and promote pentose phosphate pathway (PPP) shunt by increasing production of fructose-6-phosphate, resulting in increased production of antioxidant NADPH and nucleotide synthesis precursor, ribose-5-

phosphate (Bensaad, Cheung et al. 2009, Lui, Lau et al. 2010, Lui, Wong et al. 2011, Yin, Kosugi et al. 2012). TIGAR has also been shown to have a role in DNA damage response and repair, as evidenced by the induction of TIGAR expression with DNA damaging agent epirubicin and a decrease in DNA repair in TIGAR-knockdown cells through the regulation of the CDK5-ATM pathway (Yu, Xie et al. 2015). Consistent with the prior reports, we showed that TIGAR knockdown leads to the increase in intracellular ROS levels and DNA damage. TIGAR has also been shown to regulate cellular apoptosis and autophagy (Bensaad, Cheung et al. 2009), and confer radiotherapy resistance (Pena-Rico, Calvo-Vidal et al. 2011).

In these studies, we showed for the first time that TIGAR also plays an important role in PARP inhibitor resistance. Through a genetic screening approach and subsequent validation studies, we found that *TIGAR* knockdown leads to olaparib sensitivity in a panel of ovarian cancer cell lines. Then, we showed that *TIGAR* knockdown enhanced the olaparib-induced cytotoxicity possibly through increasing DNA damage, which might largely be attributed to increased intracellular ROS levels as a result of the decreased production of antioxidant NADPH. We hypothesize that this effect might be related to the inhibition of PPP after *TIGAR* silencing (Lee, Vousden et al. 2014). Interestingly, results from our RNA sequencing analysis indicated that *TIGAR* knockdown leads to significant downregulation of genes related to cell cycle progression. Consistently, we observed the increase in the number of cells arrested in S-phase, and this arrest was more profound in *TIGAR* knockdown cells treated with olaparib. This finding is very different from the G_2/M arrest observed in TIGAR-proficient cells treated with olaparib. It is possible that increased DNA damage in TIGAR-deficient cells prolongs the S-phase to complete the repair. The lack of nucleotides for DNA synthesis might be another factor causing the S-phase arrest since the generation of nucleotide precursor ribose-5-phosphate through PPP is inhibited in TIGAR-

knockdown cells (Bensaad, Tsuruta et al. 2006). However, further studies are needed to clarify the underlying mechanisms involved in the S-phase arrest. We also observed induction of senescence with *TIGAR* knockdown in ovarian cancer cells, implying that targeting TIGAR as a monotherapy may be sufficient to stop cancer growth and prevent cancer progression. The highest level of senescence was seen when TIGAR was efficiently depleted, suggesting that complete depletion of TIGAR might produce more profound effects. Further studies are needed to test this concept.

The most exciting finding from this study is that *TIGAR* silencing via RNAi approaches leads to downregulation of *BRCA1* expression and the FA pathway, suggesting loss of TIGAR negatively affects the HR process, leading to induction of *"BRCAness"*. This finding provides a molecular basis for enhanced sensitivity to PARP inhibitor after TIGAR knockdown because induction of *"BRCAness"* in HR-proficient cancer cells is shown to re-sensitize them to PARP inhibitor (Neri, Ren et al. 2011, Ha, Fiskus et al. 2014, Li, Karanika et al. 2017). Our finding is the first to show the potential role of TIGAR in HR pathway regulation. However, further studies are needed to investigate how the loss of TIGAR contributes to the decreased levels of *BRCA1* transcripts and the suppression HR pathway. The spheroid formation assay highlighted the potential for targeting TIGAR to enhance the therapeutic activities of PARP inhibitors. A search of TCGA databases found that aberrant *TIGAR* expression is common in various types of cancer, including testicular germ cell cancer, ovarian cancer, lower grade glioma, and non-small cell lung cancer. The higher levels of TIGAR expression are associated with a less favorable patient outcome. Together, these results suggest that targeting TIGAR might be a viable approach to treat many types of cancers. In the future, *in vivo* study would be needed to evaluate the therapeutic effects of combing PARP inhibitor with inhibition of TIGAR.

Overall, our studies showed an exciting avenue to develop new combination therapies to induce *"BRCAness"* and thus enhance the efficacy of PARP inhibitor in those tumor cells with acquired or primary resistance. We have shown that FOXM1 plays an important role in adaptive response to PARP inhibitor and contributes to PARP inhibitor resistance. Therefore, inhibition of FOXM1 represents a potential strategy to overcome PARP inhibitor resistance. We also demonstrated that FOXM1 coordinates with c-MYC to regulate HR genes, such as *BRCA1, RAD51,* and *BRIP1*, and that BET bromodomain inhibitor can induce *"BRCAness"* and enhance the activity of PARP inhibitors. In addition, we showed the great potential of CRISPR/Cas9-based functional genetic screen to identify novel modifiers of PARP inhibitor response. We identified TIGAR as a novel modifier of HR pathway, and we showed that TIGAR knockdown enhances the sensitivity of ovarian cancer cells to PARP inhibitor olaparib. Our studies provided a new understanding of potential mechanisms contributing to PARP inhibitor sensitivity and suggest TIGAR as a new therapeutic target to explore for advancement of combination therapies to enhance PARP inhibitor therapeutic effects.

5.2 Future directions

As I mentioned in Chapter 1, various mechanisms have been suggested to contribute to PARP inhibitor resistance, among them restoration or enhancement of HR function is well recongnized and the most extensively studied. In this dissertaion, my studies mainly focused on new strategies to enhance the effect of PARP inhibitors by identifying factors that impair HR pathway. Besides HR repair, BRCA1 and BRCA2 were also shown to be involved in regulating replication stress (Pathania, Nguyen et al. 2011, Schlacher, Christ et al. 2011, Schlacher, Wu et al. 2012, Ying, Hamdy et al. 2012, Pathania, Bade et al. 2014, Ray Chaudhuri, Callen et al. 2016). Emerging evidence show that the enhancement of replication fork protection serves as an mechanism for

PARP inhibitor resistance in BRCA1/2-deficient cancer cells with no revertant mutations in *BRCA* genes (Ray Chaudhuri, Callen et al. 2016, Rondinelli, Gogola et al. 2017). RAD51 and the Fanconi anemia pathway components, such as FANCD2 and FANCD1/BRCA2, were shown to increase replication fork stability (Schlacher, Wu et al. 2012). Since data from our studies suggest FOXM1 and c-MYC positively regulate *RDA51* and *BRCA2*, it would be interesting to test the potential impact of FOXM1 and c-MYC on replication fork stability. In addition, our data showed that *TIGAR* knockdown leads to S-phase arest, and downregulation of *BRCA1* and the FA pathway. It would be informative to study the impact of TIGAR deficiency on replication stress, especially on replication fork stability, which might provide us additional mechanistic explanation on the enhanced sensitivity of TIGAR-deficient cells to PARP inhibitor.

Moreover, it would be informative to find answers for the folowing questions arising from the results of my studies. First, the enhanced effect of PARP inhibitor in thiostrepton-treated cells might not depend only on the downregulation of HR genes. Thiostrepton also functioned as a proteasome inhibitor (Aminake, Schoof et al. 2011, Gartel 2011) and induces intracellular ROS production (Qiao, Lamore et al. 2012). Studies have shown that proteasome inhibitors can sensitize cancer cells to PARP inhibitor (Neri, Ren et al. 2011, Gu, Bouwman et al. 2014). Therefore, the effect that we observed with thiostrepton may be the combined effects on thiostrepton on multiple cellular targets rather than its specific effect on FOXM1 inhibition. In order to evaluate the exact role of FOXM1 in modifying the PARP inhibitor response, it would be important to develop and use specific FOXM1 inhibitors. In addition, *in vivo* studies should be conducted to evaluate the therapeutic index of drug combinations consisting of PARP inhibitors and specific FOXM1 inhibitors. These studies should address the extent to which the combination of FOXM1-specific inhibitor with PARP inhibitors induce *"BRCAness"* and enhance sensitivity to PARP inhibitors *in*

vivo without causing detrimental effects on immune cells and normal tissues. Second, although we showed that the suppression of both FOXM1 and c-MYC by the BET inhibitor (+)-JQ1 results in the downregulation of *BRCA1* and *RAD51* and the sensitization of resistant cancer cells to PARP inhibitor, it would be informative to show the effects is dependent on FOXM1 and c-MYC. Rescue of *BRCA1* and *RAD51* expression and resistance to PARP inhibitors in cells with enforced expression of FOXM1 and c-MYC in the presence of (+)-JQ1 will clarify the direct of FOXM1 and c-MYC in the regulation of BRCA1 and RAD51 in (+)-JQ1-treated cells. Moreover, the assessment of PARP inhibitor sensitivity in cancer cells with genetic depletion of both FOXM1 and c-MYC will further clarify the role of these proteins in PARP inhibitor sensitivity. However, the generation of mutant clones with genetic depletion of both FOXM1 and MYC may not be possible because cells may be addicted to these oncogenes. Even in cases where viable clones are generated, it is possible that such clones may have acquired adaptive responses to compensate for the loss in FOXM1 and MYC, and these adaptive responses may modify PARP inhibitor sensitivity. Therefore, inducible systems where both FOXM1 and MYC can be knockdown transiently in controlled manner may be needed to test the role of dual inhibition of FOXM1 and MYC in PARP inhibitor sensitivity. More importantly, in order to facilitate the translation of *in vitro* observations into clinic, it would be important to test the combined effects of BET inhibitor and PARP inhibitor *in vivo*. These studies should evaluate not only the pharmacokinetics of BET degraders and inhibitors in combination with PARP inhibitors but also the pharmacodynamics of these agents on inducing *"BRCAness"* in cancer cells as well as in normal cells and the potential toxicity and adverse effects of the combination. Third, we performed *in vitro* studies to demonstrate that TIGAR knockdown increases PARP inhibitor sensitivity in ovarian cancer cells, and these results suggest a therapeutic potential of targeting TIGAR to enhance effects of PARP

inhibitor. Although the clinical relevance is partially supported by the observation that TIGAR is amplified in tumor samples from TCGA studies and that higher TIGAR expression is associated with poor survival outcome in high-grade serous ovarian cancer, additional *in vivo* studies are needed to further evaluate the feasibility of targeting TIGAR. Besides genetic manipulation of *TIGAR* expression, it would be important to develop specific TIGAR inhibitors. Further studies are also needed to unveil how TIGAR downregulation leads to cellular senescence. In addition, it would be informative to perform further studies to unveil the detailed mechanisms affecting *BRCA1* expression and the FA pathway.

References

Adams, J. M., A. W. Harris, C. A. Pinkert, L. M. Corcoran, W. S. Alexander, S. Cory, R. D. Palmiter and R. L. Brinster (1985). "The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice." Nature **318**(6046): 533-538.

Adamson, B., A. Smogorzewska, F. D. Sigoillot, R. W. King and S. J. Elledge (2012). "A genome-wide homologous recombination screen identifies the RNA-binding protein RBMX as a component of the DNA-damage response." Nat Cell Biol **14**(3): 318-328.

Ahmad, R., M. Alam, M. Hasegawa, Y. Uchida, O. Al-Obaid, S. Kharbanda and D. Kufe (2017). "Targeting MUC1-C inhibits the AKT-S6K1-elF4A pathway regulating TIGAR translation in colorectal cancer." Mol Cancer **16**(1): 33.

Ahmed, A. A., D. Etemadmoghadam, J. Temple, A. G. Lynch, M. Riad, R. Sharma, C. Stewart, S. Fereday, C. Caldas, A. Defazio, D. Bowtell and J. D. Brenton (2010). "Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary." J Pathol **221**(1): 49-56.

Al-Ejeh, F., W. Shi, M. Miranda, P. T. Simpson, A. C. Vargas, S. Song, A. P. Wiegmans, A. Swarbrick, A. L. Welm, M. P. Brown, G. Chenevix-Trench, S. R. Lakhani and K. K. Khanna (2013). "Treatment of triple-negative breast cancer using anti-EGFR-directed radioimmunotherapy combined with radiosensitizing chemotherapy and PARP inhibitor." J Nucl Med **54**(6): 913-921.

Aleman, L. M., J. Doench and P. A. Sharp (2007). "Comparison of siRNA-induced off-target RNA and protein effects." RNA **13**(3): 385-395.

Alter, B. P. (2014). "Fanconi anemia and the development of leukemia." Best Pract Res Clin Haematol **27**(3-4): 214-221.

American Cancer Society. (2017). "What is ovarian cancer?", from https://www.cancer.org/cancer/ovarian-cancer/about/what-is-ovarian-cancer.html.

Aminake, M. N., S. Schoof, L. Sologub, M. Leubner, M. Kirschner, H. D. Arndt and G. Pradel (2011). "Thiostrepton and derivatives exhibit antimalarial and gametocytocidal activity by dually targeting parasite proteasome and apicoplast." Antimicrob Agents Chemother **55**(4): 1338-1348.

Antoniou, A., P. D. Pharoah, S. Narod, H. A. Risch, J. E. Eyfjord, J. L. Hopper, N. Loman, H. Olsson, O. Johannsson, A. Borg, B. Pasini, P. Radice, S. Manoukian, D. M. Eccles, N. Tang, E. Olah, H. Anton-Culver, E. Warner, J. Lubinski, J. Gronwald, B. Gorski, H. Tulinius, S. Thorlacius, H. Eerola, H. Nevanlinna, K. Syrjakoski, O. P. Kallioniemi, D. Thompson, C. Evans, J. Peto, F. Lalloo, D. G. Evans and D. F. Easton (2003). "Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies." Am J Hum Genet **72**(5): 1117-1130.

Arian-Schad, K. S., D. S. Kapp, A. Hackl, F. M. Juettner, H. Leitner, G. Porsch, M. Lahousen and H. Pickel (1990). "Radiation therapy in stage III ovarian cancer following surgery and

chemotherapy: prognostic factors, patterns of relapse, and toxicity: a preliminary report." Gynecol Oncol **39**(1): 47-55.

Armstrong, D. K., B. Bundy, L. Wenzel, H. Q. Huang, R. Baergen, S. Lele, L. J. Copeland, J. L. Walker, R. A. Burger and G. Gynecologic Oncology (2006). "Intraperitoneal cisplatin and paclitaxel in ovarian cancer." N Engl J Med **354**(1): 34-43.

Arnaudeau, C., C. Lundin and T. Helleday (2001). "DNA double-strand breaks associated with replication forks are predominantly repaired by homologous recombination involving an exchange mechanism in mammalian cells." J Mol Biol **307**(5): 1235-1245.

Ashworth, A. (2008). "Drug resistance caused by reversion mutation." Cancer Res **68**(24): 10021-10023.

Audeh, M. W., J. Carmichael, R. T. Penson, M. Friedlander, B. Powell, K. M. Bell-McGuinn, C. Scott, J. N. Weitzel, A. Oaknin, N. Loman, K. Lu, R. K. Schmutzler, U. Matulonis, M. Wickens and A. Tutt (2010). "Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial." Lancet **376**(9737): 245-251.

Auersperg, N., A. S. Wong, K. C. Choi, S. K. Kang and P. C. Leung (2001). "Ovarian surface epithelium: biology, endocrinology, and pathology." Endocr Rev **22**(2): 255-288.

Aylon, Y., B. Liefshitz and M. Kupiec (2004). "The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle." EMBO J **23**(24): 4868-4875.

Bajrami, I., J. R. Frankum, A. Konde, R. E. Miller, F. L. Rehman, R. Brough, J. Campbell, D. Sims, R. Rafiq, S. Hooper, L. Chen, I. Kozarewa, I. Assiotis, K. Fenwick, R. Natrajan, C. J. Lord and A. Ashworth (2014). "Genome-wide profiling of genetic synthetic lethality identifies CDK12 as a novel determinant of PARP1/2 inhibitor sensitivity." Cancer Res **74**(1): 287-297.

Barazzuol, L., R. Jena, N. G. Burnet, L. B. Meira, J. C. Jeynes, K. J. Kirkby and N. F. Kirkby (2013). "Evaluation of poly (ADP-ribose) polymerase inhibitor ABT-888 combined with radiotherapy and temozolomide in glioblastoma." Radiat Oncol **8**: 65.

Baselga, J., J. Cortes, S. B. Kim, S. A. Im, R. Hegg, Y. H. Im, L. Roman, J. L. Pedrini, T. Pienkowski, A. Knott, E. Clark, M. C. Benyunes, G. Ross, S. M. Swain and C. S. Group (2012). "Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer." N Engl J Med **366**(2): 109-119.

Bast, R. C., Jr., B. Hennessy and G. B. Mills (2009). "The biology of ovarian cancer: new opportunities for translation." Nat Rev Cancer **9**(6): 415-428.

Bastola, P., L. Neums, F. J. Schoenen and J. Chien (2016). "VCP inhibitors induce endoplasmic reticulum stress, cause cell cycle arrest, trigger caspase-mediated cell death and synergistically kill ovarian cancer cells in combination with Salubrinal." Mol Oncol **10**(10): 1559-1574.

Bayat Mokhtari, R., T. S. Homayouni, N. Baluch, E. Morgatskaya, S. Kumar, B. Das and H. Yeger (2017). "Combination therapy in combating cancer." Oncotarget **8**(23): 38022-38043.

Bella, L., S. Zona, G. Nestal de Moraes and E. W. Lam (2014). "FOXM1: A key oncofoetal transcription factor in health and disease." Semin Cancer Biol **29**: 32-39.

Bellamy, W. T. (1996). "P-glycoproteins and multidrug resistance." Annu Rev Pharmacol Toxicol **36**: 161-183.

Benafif, S. and M. Hall (2015). "An update on PARP inhibitors for the treatment of cancer." Onco Targets Ther **8**: 519-528.

Benedet, J. L., H. Bender, H. Jones, 3rd, H. Y. Ngan and S. Pecorelli (2000). "FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology." Int J Gynaecol Obstet **70**(2): 209-262.

Bensaad, K., E. C. Cheung and K. H. Vousden (2009). "Modulation of intracellular ROS levels by TIGAR controls autophagy." EMBO J **28**(19): 3015-3026.

Bensaad, K., A. Tsuruta, M. A. Selak, M. N. Vidal, K. Nakano, R. Bartrons, E. Gottlieb and K. H. Vousden (2006). "TIGAR, a p53-inducible regulator of glycolysis and apoptosis." Cell **126**(1): 107-120.

Berkovich, E., R. J. Monnat, Jr. and M. B. Kastan (2007). "Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair." Nat Cell Biol **9**(6): 683-690.

Bhalla, A. and M. W. Saif (2014). "PARP-inhibitors in BRCA-associated pancreatic cancer." JOP **15**(4): 340-343.

Bollag, G., P. Hirth, J. Tsai, J. Zhang, P. N. Ibrahim, H. Cho, W. Spevak, C. Zhang, Y. Zhang, G. Habets, E. A. Burton, B. Wong, G. Tsang, B. L. West, B. Powell, R. Shellooe, A. Marimuthu, H. Nguyen, K. Y. Zhang, D. R. Artis, J. Schlessinger, F. Su, B. Higgins, R. Iyer, K. D'Andrea, A. Koehler, M. Stumm, P. S. Lin, R. J. Lee, J. Grippo, I. Puzanov, K. B. Kim, A. Ribas, G. A. McArthur, J. A. Sosman, P. B. Chapman, K. T. Flaherty, X. Xu, K. L. Nathanson and K. Nolop (2010). "Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma." Nature **467**(7315): 596-599.

Bolton, K. L., G. Chenevix-Trench, C. Goh, S. Sadetzki, S. J. Ramus, B. Y. Karlan, D. Lambrechts, E. Despierre, D. Barrowdale, L. McGuffog, S. Healey, D. F. Easton, O. Sinilnikova, J. Benitez, M. J. Garcia, S. Neuhausen, M. H. Gail, P. Hartge, S. Peock, D. Frost, D. G. Evans, R. Eeles, A. K. Godwin, M. B. Daly, A. Kwong, E. S. Ma, C. Lazaro, I. Blanco, M. Montagna, E. D'Andrea, M. O. Nicoletto, S. E. Johnatty, S. K. Kjaer, A. Jensen, E. Hogdall, E. L. Goode, B. L. Fridley, J. T. Loud, M. H. Greene, P. L. Mai, A. Chetrit, F. Lubin, G. Hirsh-Yechezkel, G. Glendon, I. L. Andrulis, A. E. Toland, L. Senter, M. E. Gore, C. Gourley, C. O. Michie, H. Song, J. Tyrer, A. S. Whittemore, V. McGuire, W. Sieh, U. Kristoffersson, H. Olsson, A. Borg, D. A. Levine, L. Steele, M. S. Beattie, S. Chan, R. L. Nussbaum, K. B. Moysich, J. Gross, I. Cass, C. Walsh, A. J. Li, R. Leuchter, O. Gordon, M. Garcia-Closas, S. A. Gayther, S. J. Chanock, A. C.

Antoniou, P. D. Pharoah, Embrace, I. kConFab and N. Cancer Genome Atlas Research (2012). "Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer." JAMA **307**(4): 382-390.

Booker, M., A. A. Samsonova, Y. Kwon, I. Flockhart, S. E. Mohr and N. Perrimon (2011). "False negative rates in Drosophila cell-based RNAi screens: a case study." BMC Genomics **12**: 50.

Boone, C., H. Bussey and B. J. Andrews (2007). "Exploring genetic interactions and networks with yeast." Nat Rev Genet **8**(6): 437-449.

Bouwman, P., A. Aly, J. M. Escandell, M. Pieterse, J. Bartkova, H. van der Gulden, S. Hiddingh, M. Thanasoula, A. Kulkarni, Q. Yang, B. G. Haffty, J. Tommiska, C. Blomqvist, R. Drapkin, D. J. Adams, H. Nevanlinna, J. Bartek, M. Tarsounas, S. Ganesan and J. Jonkers (2010). "53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers." Nat Struct Mol Biol **17**(6): 688-695.

Bouwman, P. and J. Jonkers (2014). "Molecular pathways: how can BRCA-mutated tumors become resistant to PARP inhibitors?" Clin Cancer Res **20**(3): 540-547.

Bowling, B. D., N. Doudican, P. Manga and S. J. Orlow (2008). "Inhibition of mitochondrial protein translation sensitizes melanoma cells to arsenic trioxide cytotoxicity via a reactive oxygen species dependent mechanism." Cancer Chemother Pharmacol **63**(1): 37-43.

Bowtell, D. D., S. Bohm, A. A. Ahmed, P. J. Aspuria, R. C. Bast, Jr., V. Beral, J. S. Berek, M. J. Birrer, S. Blagden, M. A. Bookman, J. D. Brenton, K. B. Chiappinelli, F. C. Martins, G. Coukos, R. Drapkin, R. Edmondson, C. Fotopoulou, H. Gabra, J. Galon, C. Gourley, V. Heong, D. G. Huntsman, M. Iwanicki, B. Y. Karlan, A. Kaye, E. Lengyel, D. A. Levine, K. H. Lu, I. A. McNeish, U. Menon, S. A. Narod, B. H. Nelson, K. P. Nephew, P. Pharoah, D. J. Powell, Jr., P. Ramos, I. L. Romero, C. L. Scott, A. K. Sood, E. A. Stronach and F. R. Balkwill (2015). "Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer." Nat Rev Cancer **15**(11): 668-679.

Branzei, D. and M. Foiani (2008). "Regulation of DNA repair throughout the cell cycle." Nat Rev Mol Cell Biol **9**(4): 297-308.

Brenner, J. C., B. Ateeq, Y. Li, A. K. Yocum, Q. Cao, I. A. Asangani, S. Patel, X. Wang, H. Liang, J. Yu, N. Palanisamy, J. Siddiqui, W. Yan, X. Cao, R. Mehra, A. Sabolch, V. Basrur, R. J. Lonigro, J. Yang, S. A. Tomlins, C. A. Maher, K. S. Elenitoba-Johnson, M. Hussain, N. M. Navone, K. J. Pienta, S. Varambally, F. Y. Feng and A. M. Chinnaiyan (2011). "Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer." Cancer Cell **19**(5): 664-678.

Brenner, J. C., F. Y. Feng, S. Han, S. Patel, S. V. Goyal, L. M. Bou-Maroun, M. Liu, R. Lonigro, J. R. Prensner, S. A. Tomlins and A. M. Chinnaiyan (2012). "PARP-1 inhibition as a targeted strategy to treat Ewing's sarcoma." Cancer Res **72**(7): 1608-1613.

Bridges, C. B. (1922). "The origin of variation." Amer Nat. **56**: 51-63.

Bristow, R. E., R. S. Tomacruz, D. K. Armstrong, E. L. Trimble and F. J. Montz (2002). "Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis." J Clin Oncol **20**(5): 1248-1259.

Brose, M. S., T. R. Rebbeck, K. A. Calzone, J. E. Stopfer, K. L. Nathanson and B. L. Weber (2002). "Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program." J Natl Cancer Inst **94**(18): 1365-1372.

Brown, L. A., J. Irving, R. Parker, H. Kim, J. Z. Press, T. A. Longacre, S. Chia, A. Magliocco, N. Makretsov, B. Gilks, J. Pollack and D. Huntsman (2006). "Amplification of EMSY, a novel oncogene on 11q13, in high grade ovarian surface epithelial carcinomas." Gynecol Oncol **100**(2): 264-270.

Brummelkamp, T. R., R. Bernards and R. Agami (2002). "A system for stable expression of short interfering RNAs in mammalian cells." Science **296**(5567): 550-553.

Bryant, H. E., N. Schultz, H. D. Thomas, K. M. Parker, D. Flower, E. Lopez, S. Kyle, M. Meuth, N. J. Curtin and T. Helleday (2005). "Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase." Nature **434**(7035): 913-917.

Buchner, M., E. Park, H. Geng, L. Klemm, J. Flach, E. Passegue, H. Schjerven, A. Melnick, E. Paietta, D. Kopanja, P. Raychaudhuri and M. Muschen (2015). "Identification of FOXM1 as a therapeutic target in B-cell lineage acute lymphoblastic leukaemia." Nat Commun **6**: 6471.

Bunting, S. F., E. Callen, M. L. Kozak, J. M. Kim, N. Wong, A. J. Lopez-Contreras, T. Ludwig, R. Baer, R. B. Faryabi, A. Malhowski, H. T. Chen, O. Fernandez-Capetillo, A. D'Andrea and A. Nussenzweig (2012). "BRCA1 functions independently of homologous recombination in DNA interstrand crosslink repair." Mol Cell **46**(2): 125-135.

Bunting, S. F., E. Callen, N. Wong, H. T. Chen, F. Polato, A. Gunn, A. Bothmer, N. Feldhahn, O. Fernandez-Capetillo, L. Cao, X. Xu, C. X. Deng, T. Finkel, M. Nussenzweig, J. M. Stark and A. Nussenzweig (2010). "53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks." Cell **141**(2): 243-254.

Burger, R. A., M. F. Brady, M. A. Bookman, G. F. Fleming, B. J. Monk, H. Huang, R. S. Mannel, H. D. Homesley, J. Fowler, B. E. Greer, M. Boente, M. J. Birrer, S. X. Liang and G. Gynecologic Oncology (2011). "Incorporation of bevacizumab in the primary treatment of ovarian cancer." N Engl J Med **365**(26): 2473-2483.

Burgess, M. and S. Puhalla (2014). "BRCA 1/2-Mutation Related and Sporadic Breast and Ovarian Cancers: More Alike than Different." Front Oncol **4**: 19.

Cancer Genome Atlas Research, N. (2011). "Integrated genomic analyses of ovarian carcinoma." Nature **474**(7353): 609-615.

Cannistra, S. A. (2004). "Cancer of the ovary." N Engl J Med **351**(24): 2519-2529.

Cao, L., X. Xu, S. F. Bunting, J. Liu, R. H. Wang, L. L. Cao, J. J. Wu, T. N. Peng, J. Chen, A. Nussenzweig, C. X. Deng and T. Finkel (2009). "A selective requirement for 53BP1 in the biological response to genomic instability induced by Brca1 deficiency." Mol Cell **35**(4): 534-541.

Chapman, P. B., A. Hauschild, C. Robert, J. B. Haanen, P. Ascierto, J. Larkin, R. Dummer, C. Garbe, A. Testori, M. Maio, D. Hogg, P. Lorigan, C. Lebbe, T. Jouary, D. Schadendorf, A. Ribas, S. J. O'Day, J. A. Sosman, J. M. Kirkwood, A. M. Eggermont, B. Dreno, K. Nolop, J. Li, B. Nelson, J. Hou, R. J. Lee, K. T. Flaherty, G. A. McArthur and B.-S. Group (2011). "Improved survival with vemurafenib in melanoma with BRAF V600E mutation." N Engl J Med **364**(26): 2507-2516.

Chen, S., P. Li, J. Li, Y. Wang, Y. Du, X. Chen, W. Zang, H. Wang, H. Chu, G. Zhao and G. Zhang (2015). "MiR-144 inhibits proliferation and induces apoptosis and autophagy in lung cancer cells by targeting TIGAR." Cell Physiol Biochem **35**(3): 997-1007.

Chen, S. and G. Parmigiani (2007). "Meta-analysis of BRCA1 and BRCA2 penetrance." J Clin Oncol **25**(11): 1329-1333.

Chen, S., N. E. Sanjana, K. Zheng, O. Shalem, K. Lee, X. Shi, D. A. Scott, J. Song, J. Q. Pan, R. Weissleder, H. Lee, F. Zhang and P. A. Sharp (2015). "Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis." Cell **160**(6): 1246-1260.

Chen, V. W., B. Ruiz, J. L. Killeen, T. R. Cote, X. C. Wu and C. N. Correa (2003). "Pathology and classification of ovarian tumors." Cancer **97**(10 Suppl): 2631-2642.

Chen, Y., J. McGee, X. Chen, T. N. Doman, X. Gong, Y. Zhang, N. Hamm, X. Ma, R. E. Higgs, S. V. Bhagwat, S. Buchanan, S. B. Peng, K. A. Staschke, V. Yadav, Y. Yue and H. Kouros-Mehr (2014). "Identification of druggable cancer driver genes amplified across TCGA datasets." PLoS One **9**(5): e98293.

Cheung, E. C., D. Athineos, P. Lee, R. A. Ridgway, W. Lambie, C. Nixon, D. Strathdee, K. Blyth, O. J. Sansom and K. H. Vousden (2013). "TIGAR is required for efficient intestinal regeneration and tumorigenesis." Dev Cell **25**(5): 463-477.

Chien, J., R. Kuang, C. Landen and V. Shridhar (2013). "Platinum-Sensitive Recurrence in Ovarian Cancer: The Role of Tumor Microenvironment." Front Oncol **3**: 251.

Cho, K. R. and M. Shih Ie (2009). "Ovarian cancer." Annu Rev Pathol **4**: 287-313.

Choi, Y. H. and A. M. Yu (2014). "ABC transporters in multidrug resistance and pharmacokinetics, and strategies for drug development." Curr Pharm Des **20**(5): 793-807.

Chu, G., R. Mantin, Y. M. Shen, G. Baskett and H. Sussman (1993). "Massive cisplatin overdose by accidental substitution for carboplatin. Toxicity and management." Cancer **72**(12): 3707-3714.

Cole, M. D. and V. H. Cowling (2008). "Transcription-independent functions of MYC: regulation of translation and DNA replication." Nat Rev Mol Cell Biol **9**(10): 810-815.

Coleman, R. L., A. M. Oza, D. Lorusso, C. Aghajanian, A. Oaknin, A. Dean, N. Colombo, J. I. Weberpals, A. Clamp, G. Scambia, A. Leary, R. W. Holloway, M. A. Gancedo, P. C. Fong, J. C. Goh, D. M. O'Malley, D. K. Armstrong, J. Garcia-Donas, E. M. Swisher, A. Floquet, G. E. Konecny, I. A. McNeish, C. L. Scott, T. Cameron, L. Maloney, J. Isaacson, S. Goble, C. Grace, T. C. Harding, M. Raponi, J. Sun, K. K. Lin, H. Giordano, J. A. Ledermann and A. investigators (2017). "Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial." Lancet **390**(10106): 1949-1961.

Concannon, C. G., B. F. Koehler, C. Reimertz, B. M. Murphy, C. Bonner, N. Thurow, M. W. Ward, A. Villunger, A. Strasser, D. Kogel and J. H. Prehn (2007). "Apoptosis induced by proteasome inhibition in cancer cells: predominant role of the p53/PUMA pathway." Oncogene **26**(12): 1681-1692.

Cooley, M., P. Fang, F. Fang, K. P. Nephew and J. Chien (2015). "Molecular determinants of chemotherapy resistance in ovarian cancer." Pharmacogenomics **16**(16): 1763-1767.

Cousineau, I. and A. Belmaaza (2011). "EMSY overexpression disrupts the BRCA2/RAD51 pathway in the DNA-damage response: implications for chromosomal instability/recombination syndromes as checkpoint diseases." Mol Genet Genomics **285**(4): 325-340.

Da Costa, D., A. Agathanggelou, T. Perry, V. Weston, E. Petermann, A. Zlatanou, C. Oldreive, W. Wei, G. Stewart, J. Longman, E. Smith, P. Kearns, S. Knapp and T. Stankovic (2013). "BET inhibition as a single or combined therapeutic approach in primary paediatric B-precursor acute lymphoblastic leukaemia." Blood Cancer J **3**: e126.

Dang, C. V. (2012). "MYC on the path to cancer." Cell **149**(1): 22-35.

Dang, C. V., L. M. Resar, E. Emison, S. Kim, Q. Li, J. E. Prescott, D. Wonsey and K. Zeller (1999). "Function of the c-Myc oncogenic transcription factor." Exp Cell Res **253**(1): 63-77.

Dao, F., B. A. Schlappe, J. Tseng, J. Lester, A. M. Nick, S. K. Lutgendorf, S. McMeekin, R. L. Coleman, K. N. Moore, B. Y. Karlan, A. K. Sood and D. A. Levine (2016). "Characteristics of 10-year survivors of high-grade serous ovarian carcinoma." Gynecol Oncol **141**(2): 260-263.

David, S. S., V. L. O'Shea and S. Kundu (2007). "Base-excision repair of oxidative DNA damage." Nature **447**(7147): 941-950.

Dawson, C. C., C. Intapa and M. A. Jabra-Rizk (2011). ""Persisters": survival at the cellular level." PLoS Pathog **7**(7): e1002121.

Dawson, M. A., R. K. Prinjha, A. Dittmann, G. Giotopoulos, M. Bantscheff, W. I. Chan, S. C. Robson, C. W. Chung, C. Hopf, M. M. Savitski, C. Huthmacher, E. Gudgin, D. Lugo, S. Beinke, T. D. Chapman, E. J. Roberts, P. E. Soden, K. R. Auger, O. Mirguet, K. Doehner, R. Delwel, A. K. Burnett, P. Jeffrey, G. Drewes, K. Lee, B. J. Huntly and T. Kouzarides (2011). "Inhibition of

BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia." Nature **478**(7370): 529-533.

De Vos, M., V. Schreiber and F. Dantzer (2012). "The diverse roles and clinical relevance of PARPs in DNA damage repair: current state of the art." Biochem Pharmacol **84**(2): 137-146.

Dedes, K. J., P. M. Wilkerson, D. Wetterskog, B. Weigelt, A. Ashworth and J. S. Reis-Filho (2011). "Synthetic lethality of PARP inhibition in cancers lacking BRCA1 and BRCA2 mutations." Cell Cycle **10**(8): 1192-1199.

Deininger, M. W., J. M. Goldman, N. Lydon and J. V. Melo (1997). "The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells." Blood **90**(9): 3691-3698.

Delmore, J. E., G. C. Issa, M. E. Lemieux, P. B. Rahl, J. Shi, H. M. Jacobs, E. Kastritis, T. Gilpatrick, R. M. Paranal, J. Qi, M. Chesi, A. C. Schinzel, M. R. McKeown, T. P. Heffernan, C. R. Vakoc, P. L. Bergsagel, I. M. Ghobrial, P. G. Richardson, R. A. Young, W. C. Hahn, K. C. Anderson, A. L. Kung, J. E. Bradner and C. S. Mitsiades (2011). "BET bromodomain inhibition as a therapeutic strategy to target c-Myc." Cell **146**(6): 904-917.

Demetri, G. D., M. von Mehren, C. D. Blanke, A. D. Van den Abbeele, B. Eisenberg, P. J. Roberts, M. C. Heinrich, D. A. Tuveson, S. Singer, M. Janicek, J. A. Fletcher, S. G. Silverman, S. L. Silberman, R. Capdeville, B. Kiese, B. Peng, S. Dimitrijevic, B. J. Druker, C. Corless, C. D. Fletcher and H. Joensuu (2002). "Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors." N Engl J Med **347**(7): 472-480.

DePriest, P. D. and C. P. DeSimone (2003). "Ultrasound screening for the early detection of ovarian cancer." J Clin Oncol **21**(10 Suppl): 194s-199s.

Desoize, B. and C. Madoulet (2002). "Particular aspects of platinum compounds used at present in cancer treatment." Crit Rev Oncol Hematol **42**(3): 317-325.

Dever, L. A. and T. S. Dermody (1991). "Mechanisms of bacterial resistance to antibiotics." Arch Intern Med **151**(5): 886-895.

Dobin, A., C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson and T. R. Gingeras (2013). "STAR: ultrafast universal RNA-seq aligner." Bioinformatics **29**(1): 15-21.

Dominguez-Sola, D., C. Y. Ying, C. Grandori, L. Ruggiero, B. Chen, M. Li, D. A. Galloway, W. Gu, J. Gautier and R. Dalla-Favera (2007). "Non-transcriptional control of DNA replication by c-Myc." Nature **448**(7152): 445-451.

Drew, Y., E. A. Mulligan, W. T. Vong, H. D. Thomas, S. Kahn, S. Kyle, A. Mukhopadhyay, G. Los, Z. Hostomsky, E. R. Plummer, R. J. Edmondson and N. J. Curtin (2011). "Therapeutic potential of poly(ADP-ribose) polymerase inhibitor AG014699 in human cancers with mutated or methylated BRCA1 or BRCA2." J Natl Cancer Inst **103**(4): 334-346.

Druker, B. J., M. Talpaz, D. J. Resta, B. Peng, E. Buchdunger, J. M. Ford, N. B. Lydon, H. Kantarjian, R. Capdeville, S. Ohno-Jones and C. L. Sawyers (2001). "Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia." N Engl J Med **344**(14): 1031-1037.

Druker, B. J., S. Tamura, E. Buchdunger, S. Ohno, G. M. Segal, S. Fanning, J. Zimmermann and N. B. Lydon (1996). "Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells." Nat Med **2**(5): 561-566.

du Bois, A., H. J. Luck, W. Meier, H. P. Adams, V. Mobus, S. Costa, T. Bauknecht, B. Richter, M. Warm, W. Schroder, S. Olbricht, U. Nitz, C. Jackisch, G. Emons, U. Wagner, W. Kuhn, J. Pfisterer and G. Arbeitsgemeinschaft Gynakologische Onkologie Ovarian Cancer Study (2003). "A randomized clinical trial of cisplatin/paclitaxel versus carboplatin/paclitaxel as first-line treatment of ovarian cancer." J Natl Cancer Inst **95**(17): 1320-1329.

du Bois, A., J. Rochon, J. Pfisterer and W. J. Hoskins (2009). "Variations in institutional infrastructure, physician specialization and experience, and outcome in ovarian cancer: a systematic review." Gynecol Oncol **112**(2): 422-436.

Dumitriu, I. E., R. E. Voll, W. Kolowos, U. S. Gaipl, P. Heyder, J. R. Kalden and M. Herrmann (2004). "UV irradiation inhibits ABC transporters via generation of ADP-ribose by concerted action of poly(ADP-ribose) polymerase-1 and glycohydrolase." Cell Death Differ **11**(3): 314-320.

Dungey, F. A., K. W. Caldecott and A. J. Chalmers (2009). "Enhanced radiosensitization of human glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition of heat shock protein 90." Mol Cancer Ther **8**(8): 2243-2254.

Durmus, S., R. W. Sparidans, A. van Esch, E. Wagenaar, J. H. Beijnen and A. H. Schinkel (2015). "Breast cancer resistance protein (BCRP/ABCG2) and P-glycoprotein (P-GP/ABCB1) restrict oral availability and brain accumulation of the PARP inhibitor rucaparib (AG-014699)." Pharm Res **32**(1): 37-46.

Easton, D. F. (1999). "How many more breast cancer predisposition genes are there?" Breast Cancer Res **1**(1): 14-17.

Echeverri, C. J., P. A. Beachy, B. Baum, M. Boutros, F. Buchholz, S. K. Chanda, J. Downward, J. Ellenberg, A. G. Fraser, N. Hacohen, W. C. Hahn, A. L. Jackson, A. Kiger, P. S. Linsley, L. Lum, Y. Ma, B. Mathey-Prevot, D. E. Root, D. M. Sabatini, J. Taipale, N. Perrimon and R. Bernards (2006). "Minimizing the risk of reporting false positives in large-scale RNAi screens." Nat Methods **3**(10): 777-779.

Edmondson, R., J. J. Broglie, A. F. Adcock and L. Yang (2014). "Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors." Assay Drug Dev Technol **12**(4): 207-218.

Edwards, S. L., R. Brough, C. J. Lord, R. Natrajan, R. Vatcheva, D. A. Levine, J. Boyd, J. S. Reis-Filho and A. Ashworth (2008). "Resistance to therapy caused by intragenic deletion in BRCA2." Nature **451**(7182): 1111-1115.

Einhorn, N., K. Sjovall, R. C. Knapp, P. Hall, R. E. Scully, R. C. Bast, Jr. and V. R. Zurawski, Jr. (1992). "Prospective evaluation of serum CA 125 levels for early detection of ovarian cancer." Obstet Gynecol **80**(1): 14-18.

Etemadmoghadam, D., A. deFazio, R. Beroukhim, C. Mermel, J. George, G. Getz, R. Tothill, A. Okamoto, M. B. Raeder, P. Harnett, S. Lade, L. A. Akslen, A. V. Tinker, B. Locandro, K. Alsop, Y. E. Chiew, N. Traficante, S. Fereday, D. Johnson, S. Fox, W. Sellers, M. Urashima, H. B. Salvesen, M. Meyerson, D. Bowtell and A. S. Group (2009). "Integrated genome-wide DNA copy number and expression analysis identifies distinct mechanisms of primary chemoresistance in ovarian carcinomas." Clin Cancer Res **15**(4): 1417-1427.

Etemadmoghadam, D., B. A. Weir, G. Au-Yeung, K. Alsop, G. Mitchell, J. George, G. Australian Ovarian Cancer Study, S. Davis, A. D. D'Andrea, K. Simpson, W. C. Hahn and D. D. Bowtell (2013). "Synthetic lethality between CCNE1 amplification and loss of BRCA1." Proc Natl Acad Sci U S A **110**(48): 19489-19494.

Evers, B., R. Drost, E. Schut, M. de Bruin, E. van der Burg, P. W. Derksen, H. Holstege, X. Liu, E. van Drunen, H. B. Beverloo, G. C. Smith, N. M. Martin, A. Lau, M. J. O'Connor and J. Jonkers (2008). "Selective inhibition of BRCA2-deficient mammary tumor cell growth by AZD2281 and cisplatin." Clin Cancer Res **14**(12): 3916-3925.

Fang, P., J. A. Madden, L. Neums, R. K. Moulder, M. L. Forrest and J. Chien (2018). "Olaparib-induced Adaptive Response Is Disrupted by FOXM1 Targeting that Enhances Sensitivity to PARP Inhibition." Mol Cancer Res.

Farmer, H., N. McCabe, C. J. Lord, A. N. Tutt, D. A. Johnson, T. B. Richardson, M. Santarosa, K. J. Dillon, I. Hickson, C. Knights, N. M. Martin, S. P. Jackson, G. C. Smith and A. Ashworth (2005). "Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy." Nature **434**(7035): 917-921.

Fei, T., Y. Chen, T. Xiao, W. Li, L. Cato, P. Zhang, M. B. Cotter, M. Bowden, R. T. Lis, S. G. Zhao, Q. Wu, F. Y. Feng, M. Loda, H. H. He, X. S. Liu and M. Brown (2017). "Genome-wide CRISPR screen identifies HNRNPL as a prostate cancer dependency regulating RNA splicing." Proc Natl Acad Sci U S A **114**(26): E5207-E5215.

Ferrone, C. R., D. A. Levine, L. H. Tang, P. J. Allen, W. Jarnagin, M. F. Brennan, K. Offit and M. E. Robson (2009). "BRCA germline mutations in Jewish patients with pancreatic adenocarcinoma." J Clin Oncol **27**(3): 433-438.

Filippakopoulos, P., J. Qi, S. Picaud, Y. Shen, W. B. Smith, O. Fedorov, E. M. Morse, T. Keates, T. T. Hickman, I. Felletar, M. Philpott, S. Munro, M. R. McKeown, Y. Wang, A. L. Christie, N. West, M. J. Cameron, B. Schwartz, T. D. Heightman, N. La Thangue, C. A. French, O. Wiest, A. L. Kung, S. Knapp and J. E. Bradner (2010). "Selective inhibition of BET bromodomains." Nature **468**(7327): 1067-1073.

Finch, A., M. Beiner, J. Lubinski, H. T. Lynch, P. Moller, B. Rosen, J. Murphy, P. Ghadirian, E. Friedman, W. D. Foulkes, C. Kim-Sing, T. Wagner, N. Tung, F. Couch, D. Stoppa-Lyonnet, P. Ainsworth, M. Daly, B. Pasini, R. Gershoni-Baruch, C. Eng, O. I. Olopade, J. McLennan, B. Karlan, J. Weitzel, P. Sun, S. A. Narod and G. Hereditary Ovarian Cancer Clinical Study (2006). "Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 Mutation." JAMA **296**(2): 185-192.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver and C. C. Mello (1998). "Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans." Nature **391**(6669): 806-811.

Florea, A. M. and D. Busselberg (2011). "Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects." Cancers (Basel) **3**(1): 1351-1371.

Fojo, A. T., K. Ueda, D. J. Slamon, D. G. Poplack, M. M. Gottesman and I. Pastan (1987). "Expression of a multidrug-resistance gene in human tumors and tissues." Proc Natl Acad Sci U S A **84**(1): 265-269.

Fojo, T. and S. Bates (2013). "Mechanisms of resistance to PARP inhibitors--three and counting." Cancer Discov **3**(1): 20-23.

Fong, P. C., D. S. Boss, T. A. Yap, A. Tutt, P. Wu, M. Mergui-Roelvink, P. Mortimer, H. Swaisland, A. Lau, M. J. O'Connor, A. Ashworth, J. Carmichael, S. B. Kaye, J. H. Schellens and J. S. de Bono (2009). "Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers." N Engl J Med **361**(2): 123-134.

Fong, P. C., T. A. Yap, D. S. Boss, C. P. Carden, M. Mergui-Roelvink, C. Gourley, J. De Greve, J. Lubinski, S. Shanley, C. Messiou, R. A'Hern, A. Tutt, A. Ashworth, J. Stone, J. Carmichael, J. H. Schellens, J. S. de Bono and S. B. Kaye (2010). "Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval." J Clin Oncol **28**(15): 2512-2519.

French, C. A., C. L. Ramirez, J. Kolmakova, T. T. Hickman, M. J. Cameron, M. E. Thyne, J. L. Kutok, J. A. Toretsky, A. K. Tadavarthy, U. R. Kees, J. A. Fletcher and J. C. Aster (2008). "BRD-NUT oncoproteins: a family of closely related nuclear proteins that block epithelial differentiation and maintain the growth of carcinoma cells." Oncogene **27**(15): 2237-2242.

Gabay, M., Y. Li and D. W. Felsher (2014). "MYC activation is a hallmark of cancer initiation and maintenance." Cold Spring Harb Perspect Med **4**(6).

Gadducci, A. and M. E. Guerrieri (2017). "PARP inhibitors alone and in combination with other biological agents in homologous recombination deficient epithelial ovarian cancer: From the basic research to the clinic." Crit Rev Oncol Hematol **114**: 153-165.

Gambacorti-Passerini, C., P. le Coutre, L. Mologni, M. Fanelli, C. Bertazzoli, E. Marchesi, M. Di Nicola, A. Biondi, G. M. Corneo, D. Belotti, E. Pogliani and N. B. Lydon (1997). "Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis." Blood Cells Mol Dis **23**(3): 380-394.

Garcia, P. L., A. L. Miller, K. M. Kreitzburg, L. N. Council, T. L. Gamblin, J. D. Christein, M. J. Heslin, J. P. Arnoletti, J. H. Richardson, D. Chen, C. A. Hanna, S. L. Cramer, E. S. Yang, J. Qi, J. E. Bradner and K. J. Yoon (2016). "The BET bromodomain inhibitor JQ1 suppresses growth of pancreatic ductal adenocarcinoma in patient-derived xenograft models." Oncogene **35**(7): 833-845.

Garnett, M. J., E. J. Edelman, S. J. Heidorn, C. D. Greenman, A. Dastur, K. W. Lau, P. Greninger, I. R. Thompson, X. Luo, J. Soares, Q. Liu, F. Iorio, D. Surdez, L. Chen, R. J. Milano, G. R. Bignell, A. T. Tam, H. Davies, J. A. Stevenson, S. Barthorpe, S. R. Lutz, F. Kogera, K. Lawrence, A. McLaren-Douglas, X. Mitropoulos, T. Mironenko, H. Thi, L. Richardson, W. Zhou, F. Jewitt, T. Zhang, P. O'Brien, J. L. Boisvert, S. Price, W. Hur, W. Yang, X. Deng, A. Butler, H. G. Choi, J. W. Chang, J. Baselga, I. Stamenkovic, J. A. Engelman, S. V. Sharma, O. Delattre, J. Saez-Rodriguez, N. S. Gray, J. Settleman, P. A. Futreal, D. A. Haber, M. R. Stratton, S. Ramaswamy, U. McDermott and C. H. Benes (2012). "Systematic identification of genomic markers of drug sensitivity in cancer cells." Nature **483**(7391): 570-575.

Gartel, A. L. (2011). "Thiostrepton, proteasome inhibitors and FOXM1." Cell Cycle **10**(24): 4341-4342.

Gartel, A. L. (2013). "Thiazole Antibiotics Siomycin a and Thiostrepton Inhibit the Transcriptional Activity of FOXM1." Front Oncol **3**: 150.

Gaymes, T. J., A. M. Mohamedali, M. Patterson, N. Matto, A. Smith, A. Kulasekararaj, R. Chelliah, N. Curtin, F. Farzaneh, S. Shall and G. J. Mufti (2013). "Microsatellite instability induced mutations in DNA repair genes CtIP and MRE11 confer hypersensitivity to poly (ADP-ribose) polymerase inhibitors in myeloid malignancies." Haematologica **98**(9): 1397-1406.

Gerlinger, M. and C. Swanton (2010). "How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine." Br J Cancer **103**(8): 1139-1143.

Giaccone, G. and H. M. Pinedo (1996). "Drug Resistance." Oncologist **1**(1 & 2): 82-87.

Gilardini Montani, M. S., A. Prodosmo, V. Stagni, D. Merli, L. Monteonofrio, V. Gatti, M. P. Gentileschi, D. Barila and S. Soddu (2013). "ATM-depletion in breast cancer cells confers sensitivity to PARP inhibition." J Exp Clin Cancer Res **32**: 95.

Golding, S. E., R. N. Morgan, B. R. Adams, A. J. Hawkins, L. F. Povirk and K. Valerie (2009). "Pro-survival AKT and ERK signaling from EGFR and mutant EGFRvIII enhances DNA double-strand break repair in human glioma cells." Cancer Biol Ther **8**(8): 730-738.

Goldman, A., B. Majumder, A. Dhawan, S. Ravi, D. Goldman, M. Kohandel, P. K. Majumder and S. Sengupta (2015). "Temporally sequenced anticancer drugs overcome adaptive resistance by targeting a vulnerable chemotherapy-induced phenotypic transition." Nat Commun **6**: 6139.

Gordon, A. N. and J. Butler (2003). "Chemotherapeutic management of advanced ovarian cancer." Semin Oncol Nurs **19**(3 Suppl 1): 3-18.

Gormally, M. V., T. S. Dexheimer, G. Marsico, D. A. Sanders, C. Lowe, D. Matak-Vinkovic, S. Michael, A. Jadhav, G. Rai, D. J. Maloney, A. Simeonov and S. Balasubramanian (2014). "Suppression of the FOXM1 transcriptional programme via novel small molecule inhibition." Nat Commun **5**: 5165.

Gu, Y., P. Bouwman, D. Greco, J. Saarela, B. Yadav, J. Jonkers and S. G. Kuznetsov (2014). "Suppression of BRCA1 sensitizes cells to proteasome inhibitors." Cell Death Dis **5**: e1580.

Ha, K., W. Fiskus, D. S. Choi, S. Bhaskara, L. Cerchietti, S. G. Devaraj, B. Shah, S. Sharma, J. C. Chang, A. M. Melnick, S. Hiebert and K. N. Bhalla (2014). "Histone deacetylase inhibitor treatment induces 'BRCAness' and synergistic lethality with PARP inhibitor and cisplatin against human triple negative breast cancer cells." Oncotarget **5**(14): 5637-5650.

Halasi, M., D. P. Schraufnagel and A. L. Gartel (2009). "Wild-type p53 protects normal cells against apoptosis induced by thiostrepton." Cell Cycle **8**(17): 2850-2851.

Hammond, W. A., A. Swaika and K. Mody (2016). "Pharmacologic resistance in colorectal cancer: a review." Ther Adv Med Oncol **8**(1): 57-84.

Hanahan, D. and R. A. Weinberg (2011). "Hallmarks of cancer: the next generation." Cell **144**(5): 646-674.

Hart, T., M. Chandrashekhar, M. Aregger, Z. Steinhart, K. R. Brown, G. MacLeod, M. Mis, M. Zimmermann, A. Fradet-Turcotte, S. Sun, P. Mero, P. Dirks, S. Sidhu, F. P. Roth, O. S. Rissland, D. Durocher, S. Angers and J. Moffat (2015). "High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities." Cell **163**(6): 1515-1526.

Hartman, J. L. t., B. Garvik and L. Hartwell (2001). "Principles for the buffering of genetic variation." Science **291**(5506): 1001-1004.

Hassler, M. and A. G. Ladurner (2012). "Towards a structural understanding of PARP1 activation and related signalling ADP-ribosyl-transferases." Curr Opin Struct Biol **22**(6): 721-729.

Heacock, M. L., D. F. Stefanick, J. K. Horton and S. H. Wilson (2010). "Alkylation DNA damage in combination with PARP inhibition results in formation of S-phase-dependent double-strand breaks." DNA Repair (Amst) **9**(8): 929-936.

Hegde, N. S., D. A. Sanders, R. Rodriguez and S. Balasubramanian (2011). "The transcription factor FOXM1 is a cellular target of the natural product thiostrepton." Nat Chem **3**(9): 725-731.

Helleday, T. (2011). "The underlying mechanism for the PARP and BRCA synthetic lethality: clearing up the misunderstandings." Mol Oncol **5**(4): 387-393.

Hochegger, H., D. Dejsuphong, T. Fukushima, C. Morrison, E. Sonoda, V. Schreiber, G. Y. Zhao, A. Saberi, M. Masutani, N. Adachi, H. Koyama, G. de Murcia and S. Takeda (2006). "Parp-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells." EMBO J **25**(6): 1305-1314.

Hoeijmakers, J. H. (2001). "Genome maintenance mechanisms for preventing cancer." Nature **411**(6835): 366-374.

Holloman, W. K. (2011). "Unraveling the mechanism of BRCA2 in homologous recombination." Nat Struct Mol Biol **18**(7): 748-754.

Horton, J. K., D. F. Stefanick, J. M. Naron, P. S. Kedar and S. H. Wilson (2005). "Poly(ADP-ribose) polymerase activity prevents signaling pathways for cell cycle arrest after DNA methylating agent exposure." J Biol Chem **280**(16): 15773-15785.

Hoskins, W. J., W. P. McGuire, M. F. Brady, H. D. Homesley, W. T. Creasman, M. Berman, H. Ball and J. S. Berek (1994). "The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma." Am J Obstet Gynecol **170**(4): 974-979; discussion 979-980.

Howell, S. B., S. Zimm, M. Markman, I. S. Abramson, S. Cleary, W. E. Lucas and R. J. Weiss (1987). "Long-term survival of advanced refractory ovarian carcinoma patients with small-volume disease treated with intraperitoneal chemotherapy." J Clin Oncol **5**(10): 1607-1612.

Howlader N, N. A., Krapcho M, et al. (2014). "SEER Cancer Statistics Review, 1975-2011." National Cancer Institute.

Howlader N, N. A., Krapcho M, Miller D, Bishop K, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (1975-2014). "SEER Cancer Statistics Review." National Cancer Institute **https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017**.

Howlett, N. G., T. Taniguchi, S. Olson, B. Cox, Q. Waisfisz, C. De Die-Smulders, N. Persky, M. Grompe, H. Joenje, G. Pals, H. Ikeda, E. A. Fox and A. D. D'Andrea (2002). "Biallelic inactivation of BRCA2 in Fanconi anemia." Science **297**(5581): 606-609.

Huang, D. W., B. T. Sherman, Q. Tan, J. R. Collins, W. G. Alvord, J. Roayaei, R. Stephens, M. W. Baseler, H. C. Lane and R. A. Lempicki (2007). "The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists." Genome Biol **8**(9): R183.

Hughes-Davies, L., D. Huntsman, M. Ruas, F. Fuks, J. Bye, S. F. Chin, J. Milner, L. A. Brown, F. Hsu, B. Gilks, T. Nielsen, M. Schulzer, S. Chia, J. Ragaz, A. Cahn, L. Linger, H. Ozdag, E. Cattaneo, E. S. Jordanova, E. Schuuring, D. S. Yu, A. Venkitaraman, B. Ponder, A. Doherty, S. Aparicio, D. Bentley, C. Theillet, C. P. Ponting, C. Caldas and T. Kouzarides (2003). "EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer." Cell **115**(5): 523-535.

Huntoon, C. J., K. S. Flatten, A. E. Wahner Hendrickson, A. M. Huehls, S. L. Sutor, S. H. Kaufmann and L. M. Karnitz (2013). "ATR inhibition broadly sensitizes ovarian cancer cells to chemotherapy independent of BRCA status." Cancer Res **73**(12): 3683-3691.

Ibrahim, Y. H., C. Garcia-Garcia, V. Serra, L. He, K. Torres-Lockhart, A. Prat, P. Anton, P. Cozar, M. Guzman, J. Grueso, O. Rodriguez, M. T. Calvo, C. Aura, O. Diez, I. T. Rubio, J.

Perez, J. Rodon, J. Cortes, L. W. Ellisen, M. Scaltriti and J. Baselga (2012). "PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition." Cancer Discov **2**(11): 1036-1047.

Incorvaia, L., F. Passiglia, S. Rizzo, A. Galvano, A. Listi, N. Barraco, R. Maragliano, V. Calo, C. Natoli, M. Ciaccio, V. Bazan and A. Russo (2017). ""Back to a false normality": new intriguing mechanisms of resistance to PARP inhibitors." Oncotarget **8**(14): 23891-23904.

Ira, G., A. Pellicioli, A. Balijja, X. Wang, S. Fiorani, W. Carotenuto, G. Liberi, D. Bressan, L. Wan, N. M. Hollingsworth, J. E. Haber and M. Foiani (2004). "DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1." Nature **431**(7011): 1011-1017.

Itahana, K., J. Campisi and G. P. Dimri (2007). "Methods to detect biomarkers of cellular senescence: the senescence-associated beta-galactosidase assay." Methods Mol Biol **371**: 21-31.

Itzen, F., A. K. Greifenberg, C. A. Bosken and M. Geyer (2014). "Brd4 activates P-TEFb for RNA polymerase II CTD phosphorylation." Nucleic Acids Res **42**(12): 7577-7590.

Jackson, S. P. and J. Bartek (2009). "The DNA-damage response in human biology and disease." Nature **461**(7267): 1071-1078.

Jacobs, I. J. and U. Menon (2004). "Progress and challenges in screening for early detection of ovarian cancer." Mol Cell Proteomics **3**(4): 355-366.

Jasin, M. and R. Rothstein (2013). "Repair of strand breaks by homologous recombination." Cold Spring Harb Perspect Biol **5**(11): a012740.

Jaspers, J. E., A. Kersbergen, U. Boon, W. Sol, L. van Deemter, S. A. Zander, R. Drost, E. Wientjens, J. Ji, A. Aly, J. H. Doroshow, A. Cranston, N. M. Martin, A. Lau, M. J. O'Connor, S. Ganesan, P. Borst, J. Jonkers and S. Rottenberg (2013). "Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors." Cancer Discov **3**(1): 68-81.

Jiang, L., X. Wu, P. Wang, T. Wen, C. Yu, L. Wei and H. Chen (2015). "Targeting FoxM1 by thiostrepton inhibits growth and induces apoptosis of laryngeal squamous cell carcinoma." J Cancer Res Clin Oncol **141**(6): 971-981.

Johnson, D. S., A. Mortazavi, R. M. Myers and B. Wold (2007). "Genome-wide mapping of in vivo protein-DNA interactions." Science **316**(5830): 1497-1502.

Johnson, N., S. F. Johnson, W. Yao, Y. C. Li, Y. E. Choi, A. J. Bernhardy, Y. Wang, M. Capelletti, K. A. Sarosiek, L. A. Moreau, D. Chowdhury, A. Wickramanayake, M. I. Harrell, J. F. Liu, A. D. D'Andrea, A. Miron, E. M. Swisher and G. I. Shapiro (2013). "Stabilization of mutant BRCA1 protein confers PARP inhibitor and platinum resistance." Proc Natl Acad Sci U S A **110**(42): 17041-17046.

Joseph, E. W., C. A. Pratilas, P. I. Poulikakos, M. Tadi, W. Wang, B. S. Taylor, E. Halilovic, Y. Persaud, F. Xing, A. Viale, J. Tsai, P. B. Chapman, G. Bollag, D. B. Solit and N. Rosen (2010).

"The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner." Proc Natl Acad Sci U S A **107**(33): 14903-14908.

Junor, E. J., D. J. Hole, L. McNulty, M. Mason and J. Young (1999). "Specialist gynaecologists and survival outcome in ovarian cancer: a Scottish national study of 1866 patients." Br J Obstet Gynaecol **106**(11): 1130-1136.

Juvekar, A., L. N. Burga, H. Hu, E. P. Lunsford, Y. H. Ibrahim, J. Balmana, A. Rajendran, A. Papa, K. Spencer, C. A. Lyssiotis, C. Nardella, P. P. Pandolfi, J. Baselga, R. Scully, J. M. Asara, L. C. Cantley and G. M. Wulf (2012). "Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer." Cancer Discov **2**(11): 1048-1063.

Kaelin, W. G., Jr. (2012). "Molecular biology. Use and abuse of RNAi to study mammalian gene function." Science **337**(6093): 421-422.

Kalin, T. V., I. C. Wang, T. J. Ackerson, M. L. Major, C. J. Detrisac, V. V. Kalinichenko, A. Lyubimov and R. H. Costa (2006). "Increased levels of the FoxM1 transcription factor accelerate development and progression of prostate carcinomas in both TRAMP and LADY transgenic mice." Cancer Res **66**(3): 1712-1720.

Kalinina, O. A., S. A. Kalinin, E. W. Polack, I. Mikaelian, S. Panda, R. H. Costa and G. R. Adami (2003). "Sustained hepatic expression of FoxM1B in transgenic mice has minimal effects on hepatocellular carcinoma development but increases cell proliferation rates in preneoplastic and early neoplastic lesions." Oncogene **22**(40): 6266-6276.

Karakashev, S., H. Zhu, Y. Yokoyama, B. Zhao, N. Fatkhutdinov, A. V. Kossenkov, A. J. Wilson, F. Simpkins, D. Speicher, D. Khabele, B. G. Bitler and R. Zhang (2017). "BET Bromodomain Inhibition Synergizes with PARP Inhibitor in Epithelial Ovarian Cancer." Cell Rep **21**(12): 3398-3405.

Karin, M. (2006). "Nuclear factor-kappaB in cancer development and progression." Nature **441**(7092): 431-436.

Kedar, P. S., D. F. Stefanick, J. K. Horton and S. H. Wilson (2012). "Increased PARP-1 association with DNA in alkylation damaged, PARP-inhibited mouse fibroblasts." Mol Cancer Res **10**(3): 360-368.

Kennedy, R. D. and A. D. D'Andrea (2005). "The Fanconi Anemia/BRCA pathway: new faces in the crowd." Genes Dev **19**(24): 2925-2940.

Khalaileh, A., A. Dreazen, A. Khatib, R. Apel, A. Swisa, N. Kidess-Bassir, A. Maitra, O. Meyuhas, Y. Dor and G. Zamir (2013). "Phosphorylation of ribosomal protein S6 attenuates DNA damage and tumor suppression during development of pancreatic cancer." Cancer Res **73**(6): 1811-1820.

Kim, A., Y. Ueda, T. Naka and T. Enomoto (2012). "Therapeutic strategies in epithelial ovarian cancer." J Exp Clin Cancer Res **31**: 14.

Kim, I. M., T. Ackerson, S. Ramakrishna, M. Tretiakova, I. C. Wang, T. V. Kalin, M. L. Major, G. A. Gusarova, H. M. Yoder, R. H. Costa and V. V. Kalinichenko (2006). "The Forkhead Box m1 transcription factor stimulates the proliferation of tumor cells during development of lung cancer." Cancer Res **66**(4): 2153-2161.

Knoechel, B., J. E. Roderick, K. E. Williamson, J. Zhu, J. G. Lohr, M. J. Cotton, S. M. Gillespie, D. Fernandez, M. Ku, H. Wang, F. Piccioni, S. J. Silver, M. Jain, D. Pearson, M. J. Kluk, C. J. Ott, L. D. Shultz, M. A. Brehm, D. L. Greiner, A. Gutierrez, K. Stegmaier, A. L. Kung, D. E. Root, J. E. Bradner, J. C. Aster, M. A. Kelliher and B. E. Bernstein (2014). "An epigenetic mechanism of resistance to targeted therapy in T cell acute lymphoblastic leukemia." Nat Genet **46**(4): 364-370.

Kocher, S., T. Rieckmann, G. Rohaly, W. Y. Mansour, E. Dikomey, I. Dornreiter and J. Dahm-Daphi (2012). "Radiation-induced double-strand breaks require ATM but not Artemis for homologous recombination during S-phase." Nucleic Acids Res **40**(17): 8336-8347.

Koh, C. M., A. Sabo and E. Guccione (2016). "Targeting MYC in cancer therapy: RNA processing offers new opportunities." Bioessays **38**(3): 266-275.

Koike-Yusa, H., Y. Li, E. P. Tan, C. Velasco-Herrera Mdel and K. Yusa (2014). "Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library." Nat Biotechnol **32**(3): 267-273.

Konstantinopoulos, P. A., R. Ceccaldi, G. I. Shapiro and A. D. D'Andrea (2015). "Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer." Cancer Discov **5**(11): 1137-1154.

Koo, C. Y., K. W. Muir and E. W. Lam (2012). "FOXM1: From cancer initiation to progression and treatment." Biochim Biophys Acta **1819**(1): 28-37.

Korkut, A., W. Wang, E. Demir, B. A. Aksoy, X. Jing, E. J. Molinelli, O. Babur, D. L. Bemis, S. Onur Sumer, D. B. Solit, C. A. Pratilas and C. Sander (2015). "Perturbation biology nominates upstream-downstream drug combinations in RAF inhibitor resistant melanoma cells." Elife **4**.

Koshiyama, M., N. Matsumura and I. Konishi (2014). "Recent concepts of ovarian carcinogenesis: type I and type II." Biomed Res Int **2014**: 934261.

Kozlov, S. V., M. E. Graham, B. Jakob, F. Tobias, A. W. Kijas, M. Tanuji, P. Chen, P. J. Robinson, G. Taucher-Scholz, K. Suzuki, S. So, D. Chen and M. F. Lavin (2011). "Autophosphorylation and ATM activation: additional sites add to the complexity." J Biol Chem **286**(11): 9107-9119.

Krajewska, M., R. S. Fehrmann, E. G. de Vries and M. A. van Vugt (2015). "Regulators of homologous recombination repair as novel targets for cancer treatment." Front Genet **6**: 96.

Kristeleit, R. S., R. Shapira-Frommer, A. Oaknin, J. Balmaña, I. L. Ray-Coquard, S. Domchek, A. V. Tinker, C. M. Castro, S. Welch, A. M. Poveda, K. Bell-Mcguinn, G. Konecny, H. Giordano, L. Maloney, S. Goble, L. Rolfe and A. Oza (2016). "Clinical activity of the

poly(ADP-ribose) polymerase (PARP) inhibitor rucaparib in patients (pts) with high-grade ovarian carcinoma (HGOC) and a BRCA mutation (BRCAmut): Analysis of pooled data from Study 10 (parts 1, 2a, and 3) and ARIEL2 (parts 1 and 2)." Annals of Oncology **27**(suppl_6): 856O-856O.

Krum, S. A., E. la Rosa Dalugdugan, G. A. Miranda-Carboni and T. F. Lane (2010). "BRCA1 Forms a Functional Complex with gamma-H2AX as a Late Response to Genotoxic Stress." J Nucleic Acids **2010**.

Kunkel, T. A. and D. A. Erie (2015). "Eukaryotic Mismatch Repair in Relation to DNA Replication." Annu Rev Genet **49**: 291-313.

Kurman, R. J. and M. Shih Ie (2010). "The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory." Am J Surg Pathol **34**(3): 433-443.

Kutscher, A. H., E. V. Zegarelli, R. M. Rankow, J. Mercadante and J. D. Piro (1959). "Clinical laboratory studies on a new topical antibiotic: thiostrepton." Oral Surg Oral Med Oral Pathol **12**(8): 967-974.

Kwok, J. M., S. S. Myatt, C. M. Marson, R. C. Coombes, D. Constantinidou and E. W. Lam (2008). "Thiostrepton selectively targets breast cancer cells through inhibition of forkhead box M1 expression." Mol Cancer Ther **7**(7): 2022-2032.

Kwok, J. M., B. Peck, L. J. Monteiro, H. D. Schwenen, J. Millour, R. C. Coombes, S. S. Myatt and E. W. Lam (2010). "FOXM1 confers acquired cisplatin resistance in breast cancer cells." Mol Cancer Res **8**(1): 24-34.

Lafranchi, L., H. R. de Boer, E. G. de Vries, S. E. Ong, A. A. Sartori and M. A. van Vugt (2014). "APC/C(Cdh1) controls CtIP stability during the cell cycle and in response to DNA damage." EMBO J **33**(23): 2860-2879.

Lam, A. K., A. W. Ngan, M. H. Leung, D. C. Kwok, V. W. Liu, D. W. Chan, W. Y. Leung and K. M. Yao (2013). "FOXM1b, which is present at elevated levels in cancer cells, has a greater transforming potential than FOXM1c." Front Oncol **3**: 11.

Lam, E. W., J. J. Brosens, A. R. Gomes and C. Y. Koo (2013). "Forkhead box proteins: tuning forks for transcriptional harmony." Nat Rev Cancer **13**(7): 482-495.

Lamb, J., E. D. Crawford, D. Peck, J. W. Modell, I. C. Blat, M. J. Wrobel, J. Lerner, J. P. Brunet, A. Subramanian, K. N. Ross, M. Reich, H. Hieronymus, G. Wei, S. A. Armstrong, S. J. Haggarty, P. A. Clemons, R. Wei, S. A. Carr, E. S. Lander and T. R. Golub (2006). "The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease." Science **313**(5795): 1929-1935.

Langelier, M. F. and J. M. Pascal (2013). "PARP-1 mechanism for coupling DNA damage detection to poly(ADP-ribose) synthesis." Curr Opin Struct Biol **23**(1): 134-143.

Langelier, M. F., J. L. Planck, S. Roy and J. M. Pascal (2012). "Structural basis for DNA damage-dependent poly(ADP-ribosyl)ation by human PARP-1." Science **336**(6082): 728-732.

Lawlor, D., P. Martin, S. Busschots, J. Thery, J. J. O'Leary, B. T. Hennessy and B. Stordal (2014). "PARP Inhibitors as P-glyoprotein Substrates." J Pharm Sci **103**(6): 1913-1920.

Leder, A., P. K. Pattengale, A. Kuo, T. A. Stewart and P. Leder (1986). "Consequences of widespread deregulation of the c-myc gene in transgenic mice: multiple neoplasms and normal development." Cell **45**(4): 485-495.

Ledermann, J., P. Harter, C. Gourley, M. Friedlander, I. Vergote, G. Rustin, C. Scott, W. Meier, R. Shapira-Frommer, T. Safra, D. Matei, E. Macpherson, C. Watkins, J. Carmichael and U. Matulonis (2012). "Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer." N Engl J Med **366**(15): 1382-1392.

Ledermann, J., P. Harter, C. Gourley, M. Friedlander, I. Vergote, G. Rustin, C. L. Scott, W. Meier, R. Shapira-Frommer, T. Safra, D. Matei, A. Fielding, S. Spencer, B. Dougherty, M. Orr, D. Hodgson, J. C. Barrett and U. Matulonis (2014). "Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial." Lancet Oncol **15**(8): 852-861.

Lee, J. H. and T. T. Paull (2005). "ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex." Science **308**(5721): 551-554.

Lee, J. M., J. L. Hays, C. M. Annunziata, A. M. Noonan, L. Minasian, J. A. Zujewski, M. Yu, N. Gordon, J. Ji, T. M. Sissung, W. D. Figg, N. Azad, B. J. Wood, J. Doroshow and E. C. Kohn (2014). "Phase I/Ib study of olaparib and carboplatin in BRCA1 or BRCA2 mutation-associated breast or ovarian cancer with biomarker analyses." J Natl Cancer Inst **106**(6): dju089.

Lee, P., K. H. Vousden and E. C. Cheung (2014). "TIGAR, TIGAR, burning bright." Cancer Metab **2**(1): 1.

Lee, S. W., S. M. Park, Y. M. Kim, Y. S. Kim, E. K. Choi, D. Y. Kim, J. H. Kim, J. H. Nam and Y. T. Kim (2011). "Radiation therapy is a treatment to be considered for recurrent epithelial ovarian cancer after chemotherapy." Tumori **97**(5): 590-595.

Levra, M. G., K. A. Olaussen, S. Novello and J. C. Soria (2014). "PARP inhibitors: an interesting pathway also for non-small cell lung cancer?" Curr Pharm Des **20**(24): 3875-3882.

Levy-Lahad, E. and E. Friedman (2007). "Cancer risks among BRCA1 and BRCA2 mutation carriers." Br J Cancer **96**(1): 11-15.

Li, B. and C. N. Dewey (2011). "RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome." BMC Bioinformatics **12**: 323.

Li, G. Q., W. Z. Guo, Y. Zhang, J. J. Seng, H. P. Zhang, X. X. Ma, G. Zhang, J. Li, B. Yan, H. W. Tang, S. S. Li, L. D. Wang and S. J. Zhang (2016). "Suppression of BRD4 inhibits human

hepatocellular carcinoma by repressing MYC and enhancing BIM expression." Oncotarget **7**(3): 2462-2474.

Li, L., S. Karanika, G. Yang, J. Wang, S. Park, B. M. Broom, G. C. Manyam, W. Wu, Y. Luo, S. Basourakos, J. H. Song, G. E. Gallick, T. Karantanos, D. Korentzelos, A. K. Azad, J. Kim, P. G. Corn, A. M. Aparicio, C. J. Logothetis, P. Troncoso, T. Heffernan, C. Toniatti, H. S. Lee, J. S. Lee, X. Zuo, W. Chang, J. Yin and T. C. Thompson (2017). "Androgen receptor inhibitor-induced "BRCAness" and PARP inhibition are synthetically lethal for castration-resistant prostate cancer." Sci Signal **10**(480).

Li, M. and X. Yu (2013). "Function of BRCA1 in the DNA damage response is mediated by ADP-ribosylation." Cancer Cell **23**(5): 693-704.

Li, W., H. Xu, T. Xiao, L. Cong, M. I. Love, F. Zhang, R. A. Irizarry, J. S. Liu, M. Brown and X. S. Liu (2014). "MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens." Genome Biol **15**(12): 554.

Li, X., W. Qiu, B. Liu, R. Yao, S. Liu, Y. Yao and J. Liang (2013). "Forkhead box transcription factor 1 expression in gastric cancer: FOXM1 is a poor prognostic factor and mediates resistance to docetaxel." J Transl Med **11**: 204.

Li, X., R. Yao, L. Yue, W. Qiu, W. Qi, S. Liu, Y. Yao and J. Liang (2014). "FOXM1 mediates resistance to docetaxel in gastric cancer via up-regulating Stathmin." J Cell Mol Med **18**(5): 811-823.

Lindahl, T. and D. E. Barnes (2000). "Repair of endogenous DNA damage." Cold Spring Harb Symp Quant Biol **65**: 127-133.

Liu, J. F., W. T. Barry, M. Birrer, J. M. Lee, R. J. Buckanovich, G. F. Fleming, B. Rimel, M. K. Buss, S. Nattam, J. Hurteau, W. Luo, P. Quy, C. Whalen, L. Obermayer, H. Lee, E. P. Winer, E. C. Kohn, S. P. Ivy and U. A. Matulonis (2014). "Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study." Lancet Oncol **15**(11): 1207-1214.

Liu, L., W. Zhou, C. T. Cheng, X. Ren, G. Somlo, M. Y. Fong, A. R. Chin, H. Li, Y. Yu, Y. Xu, S. T. O'Connor, T. R. O'Connor, D. K. Ann, J. M. Stark and S. E. Wang (2014). "TGFbeta induces "BRCAness" and sensitivity to PARP inhibition in breast cancer by regulating DNA-repair genes." Mol Cancer Res **12**(11): 1597-1609.

Liu, T. and J. Huang (2016). "DNA End Resection: Facts and Mechanisms." Genomics Proteomics Bioinformatics **14**(3): 126-130.

Liu, X., E. K. Han, M. Anderson, Y. Shi, D. Semizarov, G. Wang, T. McGonigal, L. Roberts, L. Lasko, J. Palma, G. D. Zhu, T. Penning, S. Rosenberg, V. L. Giranda, Y. Luo, J. Leverson, E. F. Johnson and A. R. Shoemaker (2009). "Acquired resistance to combination treatment with temozolomide and ABT-888 is mediated by both base excision repair and homologous recombination DNA repair pathways." Mol Cancer Res **7**(10): 1686-1692.

Livraghi, L. and J. E. Garber (2015). "PARP inhibitors in the management of breast cancer: current data and future prospects." BMC Med **13**: 188.

Lord, C. J. and A. Ashworth (2013). "Mechanisms of resistance to therapies targeting BRCA-mutant cancers." Nat Med **19**(11): 1381-1388.

Lord, C. J. and A. Ashworth (2016). "BRCAness revisited." Nat Rev Cancer **16**(2): 110-120.

Lord, C. J., S. McDonald, S. Swift, N. C. Turner and A. Ashworth (2008). "A high-throughput RNA interference screen for DNA repair determinants of PARP inhibitor sensitivity." DNA Repair (Amst) **7**(12): 2010-2019.

Love, M. I., W. Huber and S. Anders (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." Genome Biol **15**(12): 550.

Loven, J., H. A. Hoke, C. Y. Lin, A. Lau, D. A. Orlando, C. R. Vakoc, J. E. Bradner, T. I. Lee and R. A. Young (2013). "Selective inhibition of tumor oncogenes by disruption of super-enhancers." Cell **153**(2): 320-334.

Lui, V. W., C. P. Lau, C. S. Cheung, K. Ho, M. H. Ng, S. H. Cheng, B. Hong, S. W. Tsao, C. M. Tsang, K. I. Lei, Y. Yamasaki, A. Mita and A. T. Chan (2010). "An RNA-directed nucleoside anti-metabolite, 1-(3-C-ethynyl-beta-d-ribo-pentofuranosyl)cytosine (ECyd), elicits antitumor effect via TP53-induced Glycolysis and Apoptosis Regulator (TIGAR) downregulation." Biochem Pharmacol **79**(12): 1772-1780.

Lui, V. W., E. Y. Wong, K. Ho, P. K. Ng, C. P. Lau, S. K. Tsui, C. M. Tsang, S. W. Tsao, S. H. Cheng, M. H. Ng, Y. K. Ng, E. K. Lam, B. Hong, K. W. Lo, T. S. Mok, A. T. Chan and G. B. Mills (2011). "Inhibition of c-Met downregulates TIGAR expression and reduces NADPH production leading to cell death." Oncogene **30**(9): 1127-1134.

Lupo, B. and L. Trusolino (2014). "Inhibition of poly(ADP-ribosyl)ation in cancer: old and new paradigms revisited." Biochim Biophys Acta **1846**(1): 201-215.

Lynch, T. J., D. W. Bell, R. Sordella, S. Gurubhagavatula, R. A. Okimoto, B. W. Brannigan, P. L. Harris, S. M. Haserlat, J. G. Supko, F. G. Haluska, D. N. Louis, D. C. Christiani, J. Settleman and D. A. Haber (2004). "Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib." N Engl J Med **350**(21): 2129-2139.

Madureira, P. A., R. Varshochi, D. Constantinidou, R. E. Francis, R. C. Coombes, K. M. Yao and E. W. Lam (2006). "The Forkhead box M1 protein regulates the transcription of the estrogen receptor alpha in breast cancer cells." J Biol Chem **281**(35): 25167-25176.

Maemondo, M., A. Inoue, K. Kobayashi, S. Sugawara, S. Oizumi, H. Isobe, A. Gemma, M. Harada, H. Yoshizawa, I. Kinoshita, Y. Fujita, S. Okinaga, H. Hirano, K. Yoshimori, T. Harada, T. Ogura, M. Ando, H. Miyazawa, T. Tanaka, Y. Saijo, K. Hagiwara, S. Morita, T. Nukiwa and G. North-East Japan Study (2010). "Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR." N Engl J Med **362**(25): 2380-2388.

Maloisel, L., F. Fabre and S. Gangloff (2008). "DNA polymerase delta is preferentially recruited during homologous recombination to promote heteroduplex DNA extension." Mol Cell Biol **28**(4): 1373-1382.

Marteijn, J. A., H. Lans, W. Vermeulen and J. H. Hoeijmakers (2014). "Understanding nucleotide excision repair and its roles in cancer and ageing." Nat Rev Mol Cell Biol **15**(7): 465-481.

Mayr, D., V. Kanitz, B. Anderegg, B. Luthardt, J. Engel, U. Lohrs, G. Amann and J. Diebold (2006). "Analysis of gene amplification and prognostic markers in ovarian cancer using comparative genomic hybridization for microarrays and immunohistochemical analysis for tissue microarrays." Am J Clin Pathol **126**(1): 101-109.

Mazin, A. V., A. A. Alexeev and S. C. Kowalczykowski (2003). "A novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament." J Biol Chem **278**(16): 14029-14036.

McCabe, N., N. C. Turner, C. J. Lord, K. Kluzek, A. Bialkowska, S. Swift, S. Giavara, M. J. O'Connor, A. N. Tutt, M. Z. Zdzienicka, G. C. Smith and A. Ashworth (2006). "Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition." Cancer Res **66**(16): 8109-8115.

McCleland, M. L., K. Mesh, E. Lorenzana, V. S. Chopra, E. Segal, C. Watanabe, B. Haley, O. Mayba, M. Yaylaoglu, F. Gnad and R. Firestein (2016). "CCAT1 is an enhancer-templated RNA that predicts BET sensitivity in colorectal cancer." J Clin Invest **126**(2): 639-652.

McCluggage, W. G. (2011). "Morphological subtypes of ovarian carcinoma: a review with emphasis on new developments and pathogenesis." Pathology **43**(5): 420-432.

Mendes-Pereira, A. M., S. A. Martin, R. Brough, A. McCarthy, J. R. Taylor, J. S. Kim, T. Waldman, C. J. Lord and A. Ashworth (2009). "Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors." EMBO Mol Med **1**(6-7): 315-322.

Merajver, S. D., T. M. Pham, R. F. Caduff, M. Chen, E. L. Poy, K. A. Cooney, B. L. Weber, F. S. Collins, C. Johnston and T. S. Frank (1995). "Somatic mutations in the BRCA1 gene in sporadic ovarian tumours." Nat Genet **9**(4): 439-443.

Mertz, J. A., A. R. Conery, B. M. Bryant, P. Sandy, S. Balasubramanian, D. A. Mele, L. Bergeron and R. J. Sims, 3rd (2011). "Targeting MYC dependence in cancer by inhibiting BET bromodomains." Proc Natl Acad Sci U S A **108**(40): 16669-16674.

Meyer, K. B. and N. E. Madias (1994). "Cisplatin nephrotoxicity." Miner Electrolyte Metab **20**(4): 201-213.

Millour, J., N. de Olano, Y. Horimoto, L. J. Monteiro, J. K. Langer, R. Aligue, N. Hajji and E. W. Lam (2011). "ATM and p53 regulate FOXM1 expression via E2F in breast cancer epirubicin treatment and resistance." Mol Cancer Ther **10**(6): 1046-1058.

Mimitou, E. P. and L. S. Symington (2009). "DNA end resection: many nucleases make light work." DNA Repair (Amst) **8**(9): 983-995.

Mirza, M. R., B. J. Monk, J. Herrstedt, A. M. Oza, S. Mahner, A. Redondo, M. Fabbro, J. A. Ledermann, D. Lorusso, I. Vergote, N. E. Ben-Baruch, C. Marth, R. Madry, R. D. Christensen, J. S. Berek, A. Dorum, A. V. Tinker, A. du Bois, A. Gonzalez-Martin, P. Follana, B. Benigno, P. Rosenberg, L. Gilbert, B. J. Rimel, J. Buscema, J. P. Balser, S. Agarwal, U. A. Matulonis and E.-O. N. Investigators (2016). "Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer." N Engl J Med **375**(22): 2154-2164.

Miura, K., K. Sakata, M. Someya, Y. Matsumoto, H. Matsumoto, A. Takahashi and M. Hareyama (2012). "The combination of olaparib and camptothecin for effective radiosensitization." Radiat Oncol **7**: 62.

Moffat, J., D. A. Grueneberg, X. Yang, S. Y. Kim, A. M. Kloepfer, G. Hinkle, B. Piqani, T. M. Eisenhaure, B. Luo, J. K. Grenier, A. E. Carpenter, S. Y. Foo, S. A. Stewart, B. R. Stockwell, N. Hacohen, W. C. Hahn, E. S. Lander, D. M. Sabatini and D. E. Root (2006). "A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen." Cell **124**(6): 1283-1298.

Monteiro, L. J., P. Khongkow, M. Kongsema, J. R. Morris, C. Man, D. Weekes, C. Y. Koo, A. R. Gomes, P. H. Pinto, V. Varghese, L. M. Kenny, R. Charles Coombes, R. Freire, R. H. Medema and E. W. Lam (2013). "The Forkhead Box M1 protein regulates BRIP1 expression and DNA damage repair in epirubicin treatment." Oncogene **32**(39): 4634-4645.

Morrison, C., E. Sonoda, N. Takao, A. Shinohara, K. Yamamoto and S. Takeda (2000). "The controlling role of ATM in homologous recombinational repair of DNA damage." EMBO J **19**(3): 463-471.

Moynahan, M. E. and M. Jasin (2010). "Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis." Nat Rev Mol Cell Biol **11**(3): 196-207.

Mukherjee, B., B. McEllin, C. V. Camacho, N. Tomimatsu, S. Sirasanagandala, S. Nannepaga, K. J. Hatanpaa, B. Mickey, C. Madden, E. Maher, D. A. Boothman, F. Furnari, W. K. Cavenee, R. M. Bachoo and S. Burma (2009). "EGFRvIII and DNA double-strand break repair: a molecular mechanism for radioresistance in glioblastoma." Cancer Res **69**(10): 4252-4259.

Munoz, D. M., P. J. Cassiani, L. Li, E. Billy, J. M. Korn, M. D. Jones, J. Golji, D. A. Ruddy, K. Yu, G. McAllister, A. DeWeck, D. Abramowski, J. Wan, M. D. Shirley, S. Y. Neshat, D. Rakiec, R. de Beaumont, O. Weber, A. Kauffmann, E. R. McDonald, 3rd, N. Keen, F. Hofmann, W. R. Sellers, T. Schmelzle, F. Stegmeier and M. R. Schlabach (2016). "CRISPR Screens Provide a Comprehensive Assessment of Cancer Vulnerabilities but Generate False-Positive Hits for Highly Amplified Genomic Regions." Cancer Discov **6**(8): 900-913.

Murai, J., S. Y. Huang, B. B. Das, A. Renaud, Y. Zhang, J. H. Doroshow, J. Ji, S. Takeda and Y. Pommier (2012). "Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors." Cancer Res **72**(21): 5588-5599.

Murai, J., Y. Zhang, J. Morris, J. Ji, S. Takeda, J. H. Doroshow and Y. Pommier (2014). "Rationale for poly(ADP-ribose) polymerase (PARP) inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition." J Pharmacol Exp Ther **349**(3): 408-416.

Myllynen, L., T. Rieckmann, J. Dahm-Daphi, U. Kasten-Pisula, C. Petersen, E. Dikomey and M. Kriegs (2011). "In tumor cells regulation of DNA double strand break repair through EGF receptor involves both NHEJ and HR and is independent of p53 and K-Ras status." Radiother Oncol **101**(1): 147-151.

Nakagawa, Y., A. S. Sedukhina, N. Okamoto, S. Nagasawa, N. Suzuki, T. Ohta, H. Hattori, M. Roche-Molina, A. J. Narvaez, A. D. Jeyasekharan, J. A. Bernal and K. Sato (2015). "NF-kappaB signaling mediates acquired resistance after PARP inhibition." Oncotarget **6**(6): 3825-3839.

Neri, P., L. Ren, K. Gratton, E. Stebner, J. Johnson, A. Klimowicz, P. Duggan, P. Tassone, A. Mansoor, D. A. Stewart, S. Lonial, L. H. Boise and N. J. Bahlis (2011). "Bortezomib-induced "BRCAness" sensitizes multiple myeloma cells to PARP inhibitors." Blood **118**(24): 6368-6379.

Ngo, V. N., R. E. Davis, L. Lamy, X. Yu, H. Zhao, G. Lenz, L. T. Lam, S. Dave, L. Yang, J. Powell and L. M. Staudt (2006). "A loss-of-function RNA interference screen for molecular targets in cancer." Nature **441**(7089): 106-110.

Nicodeme, E., K. L. Jeffrey, U. Schaefer, S. Beinke, S. Dewell, C. W. Chung, R. Chandwani, I. Marazzi, P. Wilson, H. Coste, J. White, J. Kirilovsky, C. M. Rice, J. M. Lora, R. K. Prinjha, K. Lee and A. Tarakhovsky (2010). "Suppression of inflammation by a synthetic histone mimic." Nature **468**(7327): 1119-1123.

Nijman, S. M. (2011). "Synthetic lethality: general principles, utility and detection using genetic screens in human cells." FEBS Lett **585**(1): 1-6.

Nilsson, J. A. and J. L. Cleveland (2003). "Myc pathways provoking cell suicide and cancer." Oncogene **22**(56): 9007-9021.

Noguchi, M., D. Yu, R. Hirayama, Y. Ninomiya, E. Sekine, N. Kubota, K. Ando and R. Okayasu (2006). "Inhibition of homologous recombination repair in irradiated tumor cells pretreated with Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin." Biochem Biophys Res Commun **351**(3): 658-663.

Norquist, B., K. A. Wurz, C. C. Pennil, R. Garcia, J. Gross, W. Sakai, B. Y. Karlan, T. Taniguchi and E. M. Swisher (2011). "Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas." J Clin Oncol **29**(22): 3008-3015.

Norris, R. E., P. C. Adamson, V. T. Nguyen and E. Fox (2014). "Preclinical evaluation of the PARP inhibitor, olaparib, in combination with cytotoxic chemotherapy in pediatric solid tumors." Pediatr Blood Cancer **61**(1): 145-150.

Nowsheen, S., T. Cooper, J. A. Stanley and E. S. Yang (2012). "Synthetic lethal interactions between EGFR and PARP inhibition in human triple negative breast cancer cells." PLoS One **7**(10): e46614.

O'Donovan, P. J. and D. M. Livingston (2010). "BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair." Carcinogenesis **31**(6): 961-967.

O'Sullivan, C. C., D. H. Moon, E. C. Kohn and J. M. Lee (2014). "Beyond Breast and Ovarian Cancers: PARP Inhibitors for BRCA Mutation-Associated and BRCA-Like Solid Tumors." Front Oncol **4**: 42.

Olivier, R. I., M. A. Lubsen-Brandsma, S. Verhoef and M. van Beurden (2006). "CA125 and transvaginal ultrasound monitoring in high-risk women cannot prevent the diagnosis of advanced ovarian cancer." Gynecol Oncol **100**(1): 20-26.

Oza, A. M., A. V. Tinker, A. Oaknin, R. Shapira-Frommer, I. A. McNeish, E. M. Swisher, I. Ray-Coquard, K. Bell-McGuinn, R. L. Coleman, D. M. O'Malley, A. Leary, L. M. Chen, D. Provencher, L. Ma, J. D. Brenton, G. E. Konecny, C. M. Castro, H. Giordano, L. Maloney, S. Goble, K. K. Lin, J. Sun, M. Raponi, L. Rolfe and R. S. Kristeleit (2017). "Antitumor activity and safety of the PARP inhibitor rucaparib in patients with high-grade ovarian carcinoma and a germline or somatic BRCA1 or BRCA2 mutation: Integrated analysis of data from Study 10 and ARIEL2." Gynecol Oncol **147**(2): 267-275.

Ozols, R. F., B. N. Bundy, B. E. Greer, J. M. Fowler, D. Clarke-Pearson, R. A. Burger, R. S. Mannel, K. DeGeest, E. M. Hartenbach, R. Baergen and G. Gynecologic Oncology (2003). "Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study." J Clin Oncol **21**(17): 3194-3200.

Paddock, M. N., A. T. Bauman, R. Higdon, E. Kolker, S. Takeda and A. M. Scharenberg (2011). "Competition between PARP-1 and Ku70 control the decision between high-fidelity and mutagenic DNA repair." DNA Repair (Amst) **10**(3): 338-343.

Paez, J. G., P. A. Janne, J. C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F. J. Kaye, N. Lindeman, T. J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M. J. Eck, W. R. Sellers, B. E. Johnson and M. Meyerson (2004). "EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy." Science **304**(5676): 1497-1500.

Pal, T., J. Permuth-Wey, J. A. Betts, J. P. Krischer, J. Fiorica, H. Arango, J. LaPolla, M. Hoffman, M. A. Martino, K. Wakeley, G. Wilbanks, S. Nicosia, A. Cantor and R. Sutphen (2005). "BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases." Cancer **104**(12): 2807-2816.

Palma, J. P., Y. C. Wang, L. E. Rodriguez, D. Montgomery, P. A. Ellis, G. Bukofzer, A. Niquette, X. Liu, Y. Shi, L. Lasko, G. D. Zhu, T. D. Penning, V. L. Giranda, S. H. Rosenberg, D. J. Frost and C. K. Donawho (2009). "ABT-888 confers broad in vivo activity in combination with temozolomide in diverse tumors." Clin Cancer Res **15**(23): 7277-7290.

Paques, F. and J. E. Haber (1999). "Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae." Microbiol Mol Biol Rev **63**(2): 349-404.

Parekh, H., K. Wiesen and H. Simpkins (1997). "Acquisition of taxol resistance via P-glycoprotein- and non-P-glycoprotein-mediated mechanisms in human ovarian carcinoma cells." Biochem Pharmacol **53**(4): 461-470.

Park, H. J., J. R. Carr, Z. Wang, V. Nogueira, N. Hay, A. L. Tyner, L. F. Lau, R. H. Costa and P. Raychaudhuri (2009). "FoxM1, a critical regulator of oxidative stress during oncogenesis." EMBO J **28**(19): 2908-2918.

Park, Y. Y., S. Y. Jung, N. B. Jennings, C. Rodriguez-Aguayo, G. Peng, S. R. Lee, S. B. Kim, K. Kim, S. H. Leem, S. Y. Lin, G. Lopez-Berestein, A. K. Sood and J. S. Lee (2012). "FOXM1 mediates Dox resistance in breast cancer by enhancing DNA repair." Carcinogenesis **33**(10): 1843-1853.

Parnas, O., M. Jovanovic, T. M. Eisenhaure, R. H. Herbst, A. Dixit, C. J. Ye, D. Przybylski, R. J. Platt, I. Tirosh, N. E. Sanjana, O. Shalem, R. Satija, R. Raychowdhury, P. Mertins, S. A. Carr, F. Zhang, N. Hacohen and A. Regev (2015). "A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks." Cell **162**(3): 675-686.

Patch, A. M., E. L. Christie, D. Etemadmoghadam, D. W. Garsed, J. George, S. Fereday, K. Nones, P. Cowin, K. Alsop, P. J. Bailey, K. S. Kassahn, F. Newell, M. C. Quinn, S. Kazakoff, K. Quek, C. Wilhelm-Benartzi, E. Curry, H. S. Leong, G. Australian Ovarian Cancer Study, A. Hamilton, L. Mileshkin, G. Au-Yeung, C. Kennedy, J. Hung, Y. E. Chiew, P. Harnett, M. Friedlander, M. Quinn, J. Pyman, S. Cordner, P. O'Brien, J. Leditschke, G. Young, K. Strachan, P. Waring, W. Azar, C. Mitchell, N. Traficante, J. Hendley, H. Thorne, M. Shackleton, D. K. Miller, G. M. Arnau, R. W. Tothill, T. P. Holloway, T. Semple, I. Harliwong, C. Nourse, E. Nourbakhsh, S. Manning, S. Idrisoglu, T. J. Bruxner, A. N. Christ, B. Poudel, O. Holmes, M. Anderson, C. Leonard, A. Lonie, N. Hall, S. Wood, D. F. Taylor, Q. Xu, J. L. Fink, N. Waddell, R. Drapkin, E. Stronach, H. Gabra, R. Brown, A. Jewell, S. H. Nagaraj, E. Markham, P. J. Wilson, J. Ellul, O. McNally, M. A. Doyle, R. Vedururu, C. Stewart, E. Lengyel, J. V. Pearson, N. Waddell, A. deFazio, S. M. Grimmond and D. D. Bowtell (2015). "Whole-genome characterization of chemoresistant ovarian cancer." Nature **521**(7553): 489-494.

Patel, A. G., J. N. Sarkaria and S. H. Kaufmann (2011). "Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells." Proc Natl Acad Sci U S A **108**(8): 3406-3411.

Pathania, S., S. Bade, M. Le Guillou, K. Burke, R. Reed, C. Bowman-Colin, Y. Su, D. T. Ting, K. Polyak, A. L. Richardson, J. Feunteun, J. E. Garber and D. M. Livingston (2014). "BRCA1 haploinsufficiency for replication stress suppression in primary cells." Nat Commun **5**: 5496.

Pathania, S., J. Nguyen, S. J. Hill, R. Scully, G. O. Adelmant, J. A. Marto, J. Feunteun and D. M. Livingston (2011). "BRCA1 is required for postreplication repair after UV-induced DNA damage." Mol Cell **44**(2): 235-251.

Paull, T. T., E. P. Rogakou, V. Yamazaki, C. U. Kirchgessner, M. Gellert and W. M. Bonner (2000). "A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage." Curr Biol **10**(15): 886-895.

Pena-Rico, M. A., M. N. Calvo-Vidal, R. Villalonga-Planells, F. Martinez-Soler, P. Gimenez-Bonafe, A. Navarro-Sabate, A. Tortosa, R. Bartrons and A. Manzano (2011). "TP53 induced glycolysis and apoptosis regulator (TIGAR) knockdown results in radiosensitization of glioma cells." Radiother Oncol **101**(1): 132-139.

Pennington, K. P., T. Walsh, M. I. Harrell, M. K. Lee, C. C. Pennil, M. H. Rendi, A. Thornton, B. M. Norquist, S. Casadei, A. S. Nord, K. J. Agnew, C. C. Pritchard, S. Scroggins, R. L. Garcia, M. C. King and E. M. Swisher (2014). "Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas." Clin Cancer Res **20**(3): 764-775.

Pettitt, S. J., D. B. Krastev, I. Brandsma, A. Drean, F. Song, R. Aleksandrov, M. I. Harrell, M. Menon, R. Brough, J. Campbell, J. Frankum, M. Ranes, H. N. Pemberton, R. Rafiq, K. Fenwick, A. Swain, S. Guettler, J.-M. Lee, E. M. Swisher, S. Stoynov, K. Yusa, A. Ashworth and C. J. Lord (2017). "Genome-wide and high-density CRISPR-Cas9 screens identify point mutations in PARP1 causing PARP inhibitor resistance." bioRxiv.

Pettitt, S. J., F. L. Rehman, I. Bajrami, R. Brough, F. Wallberg, I. Kozarewa, K. Fenwick, I. Assiotis, L. Chen, J. Campbell, C. J. Lord and A. Ashworth (2013). "A genetic screen using the PiggyBac transposon in haploid cells identifies Parp1 as a mediator of olaparib toxicity." PLoS One **8**(4): e61520.

Pfeiffer, P., W. Goedecke and G. Obe (2000). "Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations." Mutagenesis **15**(4): 289-302.

Pfisterer, J. and J. A. Ledermann (2006). "Management of platinum-sensitive recurrent ovarian cancer." Semin Oncol **33**(2 Suppl 6): S12-16.

Pisco, A. O., A. Brock, J. Zhou, A. Moor, M. Mojtahedi, D. Jackson and S. Huang (2013). "Non-Darwinian dynamics in therapy-induced cancer drug resistance." Nat Commun **4**: 2467.

Plummer, R., P. Lorigan, N. Steven, L. Scott, M. R. Middleton, R. H. Wilson, E. Mulligan, N. Curtin, D. Wang, R. Dewji, A. Abbattista, J. Gallo and H. Calvert (2013). "A phase II study of the potent PARP inhibitor, Rucaparib (PF-01367338, AG014699), with temozolomide in patients with metastatic melanoma demonstrating evidence of chemopotentiation." Cancer Chemother Pharmacol **71**(5): 1191-1199.

Prat, J. (2012). "Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features." Virchows Arch **460**(3): 237-249.

Prat, J., H. Belhadj, J. Berek, A. Bermudez, N. Bhatla, J. Cain, L. Denny, K. Fujiwara, N. Hacker, E. Avall-Lundqvist, D. Mutch, F. Odicino, S. Pecorelli, M. Quinn, M. A. Seoud, S. K. Shrivastava and F. C. o. G. Oncology (2015). "Abridged republication of FIGO's staging

classification for cancer of the ovary, fallopian tube, and peritoneum." Eur J Gynaecol Oncol **36**(4): 367-369.

Prat, J. and F. C. o. G. Oncology (2015). "Abridged republication of FIGO's staging classification for cancer of the ovary, fallopian tube, and peritoneum." Cancer **121**(19): 3452-3454.

Prevo, R., E. Fokas, P. M. Reaper, P. A. Charlton, J. R. Pollard, W. G. McKenna, R. J. Muschel and T. B. Brunner (2012). "The novel ATR inhibitor VE-821 increases sensitivity of pancreatic cancer cells to radiation and chemotherapy." Cancer Biol Ther **13**(11): 1072-1081.

Qiao, S., S. D. Lamore, C. M. Cabello, J. L. Lesson, J. L. Munoz-Rodriguez and G. T. Wondrak (2012). "Thiostrepton is an inducer of oxidative and proteotoxic stress that impairs viability of human melanoma cells but not primary melanocytes." Biochem Pharmacol **83**(9): 1229-1240.

Queitsch, C., T. A. Sangster and S. Lindquist (2002). "Hsp90 as a capacitor of phenotypic variation." Nature **417**(6889): 618-624.

Quinn, B. A., R. Dash, S. Sarkar, B. Azab, P. Bhoopathi, S. K. Das, L. Emdad, J. Wei, M. Pellecchia, D. Sarkar and P. B. Fisher (2015). "Pancreatic Cancer Combination Therapy Using a BH3 Mimetic and a Synthetic Tetracycline." Cancer Res **75**(11): 2305-2315.

Radhakrishnan, S. K., U. G. Bhat, D. E. Hughes, I. C. Wang, R. H. Costa and A. L. Gartel (2006). "Identification of a chemical inhibitor of the oncogenic transcription factor forkhead box M1." Cancer Res **66**(19): 9731-9735.

Ramakrishnan Geethakumari, P., M. J. Schiewer, K. E. Knudsen and W. K. Kelly (2017). "PARP Inhibitors in Prostate Cancer." Curr Treat Options Oncol **18**(6): 37.

Ray Chaudhuri, A., E. Callen, X. Ding, E. Gogola, A. A. Duarte, J. E. Lee, N. Wong, V. Lafarga, J. A. Calvo, N. J. Panzarino, S. John, A. Day, A. V. Crespo, B. Shen, L. M. Starnes, J. R. de Ruiter, J. A. Daniel, P. A. Konstantinopoulos, D. Cortez, S. B. Cantor, O. Fernandez-Capetillo, K. Ge, J. Jonkers, S. Rottenberg, S. K. Sharan and A. Nussenzweig (2016). "Replication fork stability confers chemoresistance in BRCA-deficient cells." Nature **535**(7612): 382-387.

Raychaudhuri, P. and H. J. Park (2011). "FoxM1: a master regulator of tumor metastasis." Cancer Res **71**(13): 4329-4333.

Rebbeck, T. R., T. M. Friebel, E. Friedman, U. Hamann, D. Huo, A. Kwong, E. Olah, O. I. Olopade, A. R. Solano, S. H. Teo, M. Thomassen, J. N. Weitzel, T. L. Chan, F. J. Couch, D. E. Goldgar, T. A. Kruse, E. I. Palmero, S. K. Park, D. Torres, E. J. van Rensburg, L. McGuffog, M. T. Parsons, G. Leslie, C. M. Aalfs, J. Abugattas, J. Adlard, S. Agata, K. Aittomaki, L. Andrews, I. L. Andrulis, A. Arason, N. Arnold, B. K. Arun, E. Asseryanis, L. Auerbach, J. Azzollini, J. Balmana, M. Barile, R. B. Barkardottir, D. Barrowdale, J. Benitez, A. Berger, R. Berger, A. M. Blanco, K. R. Blazer, M. J. Blok, V. Bonadona, B. Bonanni, A. R. Bradbury, C. Brewer, B. Buecher, S. S. Buys, T. Caldes, A. Caliebe, M. A. Caligo, I. Campbell, S. M. Caputo, J. Chiquette, W. K. Chung, K. B. M. Claes, J. M. Collee, J. Cook, R. Davidson, M. de la Hoya, K. De Leeneer, A. de Pauw, C. Delnatte, O. Diez, Y. C. Ding, N. Ditsch, S. M. Domchek, C. M.

Dorfling, C. Velazquez, B. Dworniczak, J. Eason, D. F. Easton, R. Eeles, H. Ehrencrona, B. Ejlertsen, Embrace, C. Engel, S. Engert, D. G. Evans, L. Faivre, L. Feliubadalo, S. F. Ferrer, L. Foretova, J. Fowler, D. Frost, H. C. R. Galvao, P. A. Ganz, J. Garber, M. Gauthier-Villars, A. Gehrig, G. S. Collaborators, A. M. Gerdes, P. Gesta, G. Giannini, S. Giraud, G. Glendon, A. K. Godwin, M. H. Greene, J. Gronwald, A. Gutierrez-Barrera, E. Hahnen, J. Hauke, Hebon, A. Henderson, J. Hentschel, F. B. L. Hogervorst, E. Honisch, E. N. Imyanitov, C. Isaacs, L. Izatt, A. Izquierdo, A. Jakubowska, P. James, R. Janavicius, U. B. Jensen, E. M. John, J. Vijai, K. Kaczmarek, B. Y. Karlan, K. Kast, K. Investigators, S. W. Kim, I. Konstantopoulou, J. Korach, Y. Laitman, A. Lasa, C. Lasset, C. Lazaro, A. Lee, M. H. Lee, J. Lester, F. Lesueur, A. Liljegren, N. M. Lindor, M. Longy, J. T. Loud, K. H. Lu, J. Lubinski, E. Machackova, S. Manoukian, V. Mari, C. Martinez-Bouzas, Z. Matrai, N. Mebirouk, H. E. J. Meijers-Heijboer, A. Meindl, A. R. Mensenkamp, U. Mickys, A. Miller, M. Montagna, K. B. Moysich, A. M. Mulligan, J. Musinsky, S. L. Neuhausen, H. Nevanlinna, J. Ngeow, H. P. Nguyen, D. Niederacher, H. R. Nielsen, F. C. Nielsen, R. L. Nussbaum, K. Offit, A. Ofverholm, K. R. Ong, A. Osorio, L. Papi, J. Papp, B. Pasini, I. S. Pedersen, A. Peixoto, N. Peruga, P. Peterlongo, E. Pohl, N. Pradhan, K. Prajzendanc, F. Prieur, P. Pujol, P. Radice, S. J. Ramus, J. Rantala, M. U. Rashid, K. Rhiem, M. Robson, G. C. Rodriguez, M. T. Rogers, V. Rudaitis, A. Y. Schmidt, R. K. Schmutzler, L. Senter, P. D. Shah, P. Sharma, L. E. Side, J. Simard, C. F. Singer, A. B. Skytte, T. P. Slavin, K. Snape, H. Sobol, M. Southey, L. Steele, D. Steinemann, G. Sukiennicki, C. Sutter, C. I. Szabo, Y. Y. Tan, M. R. Teixeira, M. B. Terry, A. Teule, A. Thomas, D. L. Thull, M. Tischkowitz, S. Tognazzo, A. E. Toland, S. Topka, A. H. Trainer, N. Tung, C. J. van Asperen, A. H. van der Hout, L. E. van der Kolk, R. B. van der Luijt, M. Van Heetvelde, L. Varesco, R. Varon-Mateeva, A. Vega, C. Villarreal-Garza, A. von Wachenfeldt, L. Walker, S. Wang-Gohrke, B. Wappenschmidt, B. H. F. Weber, D. Yannoukakos, S. Y. Yoon, C. Zanzottera, J. Zidan, K. K. Zorn, C. G. Hutten Selkirk, P. J. Hulick, G. Chenevix-Trench, A. B. Spurdle, A. C. Antoniou and K. L. Nathanson (2018). "Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations." Hum Mutat.

Reid, B. M., J. B. Permuth and T. A. Sellers (2017). "Epidemiology of ovarian cancer: a review." Cancer Biol Med **14**(1): 9-32.

Richter, K. and J. Buchner (2001). "Hsp90: chaperoning signal transduction." J Cell Physiol **188**(3): 281-290.

Rodon, J., M. D. Iniesta and K. Papadopoulos (2009). "Development of PARP inhibitors in oncology." Expert Opin Investig Drugs **18**(1): 31-43.

Rondinelli, B., E. Gogola, H. Yucel, A. A. Duarte, M. van de Ven, R. van der Sluijs, P. A. Konstantinopoulos, J. Jonkers, R. Ceccaldi, S. Rottenberg and A. D. D'Andrea (2017). "EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation." Nat Cell Biol **19**(11): 1371-1378.

Ross, J. S., S. M. Ali, K. Wang, G. Palmer, R. Yelensky, D. Lipson, V. A. Miller, D. Zajchowski, L. K. Shawver and P. J. Stephens (2013). "Comprehensive genomic profiling of epithelial ovarian cancer by next generation sequencing-based diagnostic assay reveals new routes to targeted therapies." Gynecol Oncol **130**(3): 554-559.

Rothkamm, K., I. Kruger, L. H. Thompson and M. Lobrich (2003). "Pathways of DNA double-strand break repair during the mammalian cell cycle." Mol Cell Biol **23**(16): 5706-5715.

Rottenberg, S., J. E. Jaspers, A. Kersbergen, E. van der Burg, A. O. Nygren, S. A. Zander, P. W. Derksen, M. de Bruin, J. Zevenhoven, A. Lau, R. Boulter, A. Cranston, M. J. O'Connor, N. M. Martin, P. Borst and J. Jonkers (2008). "High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs." Proc Natl Acad Sci U S A **105**(44): 17079-17084.

Rutherford, S. L. and S. Lindquist (1998). "Hsp90 as a capacitor for morphological evolution." Nature **396**(6709): 336-342.

Saintigny, Y., F. Delacote, G. Vares, F. Petitot, S. Lambert, D. Averbeck and B. S. Lopez (2001). "Characterization of homologous recombination induced by replication inhibition in mammalian cells." EMBO J **20**(14): 3861-3870.

Sakai, W., E. M. Swisher, B. Y. Karlan, M. K. Agarwal, J. Higgins, C. Friedman, E. Villegas, C. Jacquemont, D. J. Farrugia, F. J. Couch, N. Urban and T. Taniguchi (2008). "Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers." Nature **451**(7182): 1116-1120.

Salani, R., R. J. Kurman, R. Giuntoli, 2nd, G. Gardner, R. Bristow, T. L. Wang and I. M. Shih (2008). "Assessment of TP53 mutation using purified tissue samples of ovarian serous carcinomas reveals a higher mutation rate than previously reported and does not correlate with drug resistance." Int J Gynecol Cancer **18**(3): 487-491.

Saleh-Gohari, N., H. E. Bryant, N. Schultz, K. M. Parker, T. N. Cassel and T. Helleday (2005). "Spontaneous homologous recombination is induced by collapsed replication forks that are caused by endogenous DNA single-strand breaks." Mol Cell Biol **25**(16): 7158-7169.

Samol, J., M. Ranson, E. Scott, E. Macpherson, J. Carmichael, A. Thomas and J. Cassidy (2012). "Safety and tolerability of the poly(ADP-ribose) polymerase (PARP) inhibitor, olaparib (AZD2281) in combination with topotecan for the treatment of patients with advanced solid tumors: a phase I study." Invest New Drugs **30**(4): 1493-1500.

Sanjana, N. E., O. Shalem and F. Zhang (2014). "Improved vectors and genome-wide libraries for CRISPR screening." Nat Methods **11**(8): 783-784.

Sanjana, N. E., O. Shalem and F. Zhang (2014). "Improved vectors and genome-wide libraries for CRISPR screening." Nat Methods **11**(8): 783-784.

Sankaranarayanan, R. and J. Ferlay (2006). "Worldwide burden of gynaecological cancer: the size of the problem." Best Pract Res Clin Obstet Gynaecol **20**(2): 207-225.

Sawyer, S. L., L. Tian, M. Kahkonen, J. Schwartzentruber, M. Kircher, G. University of Washington Centre for Mendelian, F. C. Consortium, J. Majewski, D. A. Dyment, A. M. Innes, K. M. Boycott, L. A. Moreau, J. S. Moilanen and R. A. Greenberg (2015). "Biallelic mutations in BRCA1 cause a new Fanconi anemia subtype." Cancer Discov **5**(2): 135-142.

Schlacher, K., N. Christ, N. Siaud, A. Egashira, H. Wu and M. Jasin (2011). "Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11." Cell **145**(4): 529-542.

Schlacher, K., H. Wu and M. Jasin (2012). "A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2." Cancer Cell **22**(1): 106-116.

Schneider, A., K. Peukert, M. Eilers and F. Hanel (1997). "Association of Myc with the zinc-finger protein Miz-1 defines a novel pathway for gene regulation by Myc." Curr Top Microbiol Immunol **224**: 137-146.

Schorge, J. O., C. McCann and M. G. Del Carmen (2010). "Surgical debulking of ovarian cancer: what difference does it make?" Rev Obstet Gynecol **3**(3): 111-117.

Scott, C. L., E. M. Swisher and S. H. Kaufmann (2015). "Poly (ADP-ribose) polymerase inhibitors: recent advances and future development." J Clin Oncol **33**(12): 1397-1406.

Shah, N. and D. S. Dizon (2009). "New-generation platinum agents for solid tumors." Future Oncol **5**(1): 33-42.

Shalem, O., N. E. Sanjana, E. Hartenian, X. Shi, D. A. Scott, T. S. Mikkelsen, D. Heckl, B. L. Ebert, D. E. Root, J. G. Doench and F. Zhang (2014). "Genome-scale CRISPR-Cas9 knockout screening in human cells." Science **343**(6166): 84-87.

Shalem, O., N. E. Sanjana, E. Hartenian, X. Shi, D. A. Scott, T. Mikkelson, D. Heckl, B. L. Ebert, D. E. Root, J. G. Doench and F. Zhang (2014). "Genome-scale CRISPR-Cas9 knockout screening in human cells." Science **343**(6166): 84-87.

Sharma, S. V., D. Y. Lee, B. Li, M. P. Quinlan, F. Takahashi, S. Maheswaran, U. McDermott, N. Azizian, L. Zou, M. A. Fischbach, K. K. Wong, K. Brandstetter, B. Wittner, S. Ramaswamy, M. Classon and J. Settleman (2010). "A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations." Cell **141**(1): 69-80.

Shen, D. W., A. Fojo, J. E. Chin, I. B. Roninson, N. Richert, I. Pastan and M. M. Gottesman (1986). "Human multidrug-resistant cell lines: increased mdr1 expression can precede gene amplification." Science **232**(4750): 643-645.

Shepherd, J. H. (1989). "Revised FIGO staging for gynaecological cancer." Br J Obstet Gynaecol **96**(8): 889-892.

Shi, J. and C. R. Vakoc (2014). "The mechanisms behind the therapeutic activity of BET bromodomain inhibition." Mol Cell **54**(5): 728-736.

Shih Ie, M. and R. J. Kurman (2004). "Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis." Am J Pathol **164**(5): 1511-1518.

Shiloh, Y. (2003). "ATM and related protein kinases: safeguarding genome integrity." Nat Rev Cancer **3**(3): 155-168.

Shu, S., C. Y. Lin, H. H. He, R. M. Witwicki, D. P. Tabassum, J. M. Roberts, M. Janiszewska, S. J. Huh, Y. Liang, J. Ryan, E. Doherty, H. Mohammed, H. Guo, D. G. Stover, M. B. Ekram, J. Brown, C. D'Santos, I. E. Krop, D. Dillon, M. McKeown, C. Ott, J. Qi, M. Ni, P. K. Rao, M. Duarte, S. Y. Wu, C. M. Chiang, L. Anders, R. A. Young, E. Winer, A. Letai, W. T. Barry, J. S. Carroll, H. Long, M. Brown, X. S. Liu, C. A. Meyer, J. E. Bradner and K. Polyak (2016). "Response and resistance to BET bromodomain inhibitors in triple-negative breast cancer." Nature **529**(7586): 413-417.

Siegel, R. L., K. D. Miller and A. Jemal (2015). "Cancer statistics, 2015." CA Cancer J Clin **65**(1): 5-29.

Silver, D. P. and D. M. Livingston (2012). "Mechanisms of BRCA1 tumor suppression." Cancer Discov **2**(8): 679-684.

Slabicki, M., M. Theis, D. B. Krastev, S. Samsonov, E. Mundwiller, M. Junqueira, M. Paszkowski-Rogacz, J. Teyra, A. K. Heninger, I. Poser, F. Prieur, J. Truchetto, C. Confavreux, C. Marelli, A. Durr, J. P. Camdessanche, A. Brice, A. Shevchenko, M. T. Pisabarro, G. Stevanin and F. Buchholz (2010). "A genome-scale DNA repair RNAi screen identifies SPG48 as a novel gene associated with hereditary spastic paraplegia." PLoS Biol **8**(6): e1000408.

Slamon, D., W. Eiermann, N. Robert, T. Pienkowski, M. Martin, M. Press, J. Mackey, J. Glaspy, A. Chan, M. Pawlicki, T. Pinter, V. Valero, M. C. Liu, G. Sauter, G. von Minckwitz, F. Visco, V. Bee, M. Buyse, B. Bendahmane, I. Tabah-Fisch, M. A. Lindsay, A. Riva, J. Crown and G. Breast Cancer International Research (2011). "Adjuvant trastuzumab in HER2-positive breast cancer." N Engl J Med **365**(14): 1273-1283.

Smith, M. A., O. A. Hampton, C. P. Reynolds, M. H. Kang, J. M. Maris, R. Gorlick, E. A. Kolb, R. Lock, H. Carol, S. T. Keir, J. Wu, R. T. Kurmasheva, D. A. Wheeler and P. J. Houghton (2015). "Initial testing (stage 1) of the PARP inhibitor BMN 673 by the pediatric preclinical testing program: PALB2 mutation predicts exceptional in vivo response to BMN 673." Pediatr Blood Cancer **62**(1): 91-98.

Song, T., Y. Y. Lee, C. H. Choi, T. J. Kim, J. W. Lee, D. S. Bae and B. G. Kim (2013). "Histologic distribution of borderline ovarian tumors worldwide: a systematic review." J Gynecol Oncol **24**(1): 44-51.

Sorensen, C. S., L. T. Hansen, J. Dziegielewski, R. G. Syljuasen, C. Lundin, J. Bartek and T. Helleday (2005). "The cell-cycle checkpoint kinase Chk1 is required for mammalian homologous recombination repair." Nat Cell Biol **7**(2): 195-201.

Sousa, F. G., R. Matuo, D. G. Soares, A. E. Escargueil, J. A. Henriques, A. K. Larsen and J. Saffi (2012). "PARPs and the DNA damage response." Carcinogenesis **33**(8): 1433-1440.

Stordal, B., K. Timms, A. Farrelly, D. Gallagher, S. Busschots, M. Renaud, J. Thery, D. Williams, J. Potter, T. Tran, G. Korpanty, M. Cremona, M. Carey, J. Li, Y. Li, O. Aslan, J. J. O'Leary, G. B. Mills and B. T. Hennessy (2013). "BRCA1/2 mutation analysis in 41 ovarian cell lines reveals only one functionally deleterious BRCA1 mutation." Mol Oncol **7**(3): 567-579.

Strom, C. E., F. Johansson, M. Uhlen, C. A. Szigyarto, K. Erixon and T. Helleday (2011). "Poly (ADP-ribose) polymerase (PARP) is not involved in base excision repair but PARP inhibition traps a single-strand intermediate." Nucleic Acids Res **39**(8): 3166-3175.

Sun, C. K., F. Zhang, T. Xiang, Q. Chen, T. K. Pandita, Y. Huang, M. C. Hu and Q. Yang (2014). "Phosphorylation of ribosomal protein S6 confers PARP inhibitor resistance in BRCA1-deficient cancers." Oncotarget **5**(10): 3375-3385.

Sun, L. and P. Gao (2017). "Small molecules remain on target for c-Myc." Elife **6**.

Sung, P. and H. Klein (2006). "Mechanism of homologous recombination: mediators and helicases take on regulatory functions." Nat Rev Mol Cell Biol **7**(10): 739-750.

Symington, L. S. and J. Gautier (2011). "Double-strand break end resection and repair pathway choice." Annu Rev Genet **45**: 247-271.

Tan, Y., P. Raychaudhuri and R. H. Costa (2007). "Chk2 mediates stabilization of the FoxM1 transcription factor to stimulate expression of DNA repair genes." Mol Cell Biol **27**(3): 1007-1016.

Tapodi, A., B. Debreceni, K. Hanto, Z. Bognar, I. Wittmann, F. Gallyas, Jr., G. Varbiro and B. Sumegi (2005). "Pivotal role of Akt activation in mitochondrial protection and cell survival by poly(ADP-ribose)polymerase-1 inhibition in oxidative stress." J Biol Chem **280**(42): 35767-35775.

Tassi, R. A., P. Todeschini, E. R. Siegel, S. Calza, P. Cappella, L. Ardighieri, M. Cadei, M. Bugatti, C. Romani, E. Bandiera, L. Zanotti, L. Tassone, D. Guarino, C. Santonocito, E. D. Capoluongo, L. Beltrame, E. Erba, S. Marchini, M. D'Incalci, C. Donzelli, A. D. Santin, S. Pecorelli, E. Sartori, E. Bignotti, F. Odicino and A. Ravaggi (2017). "FOXM1 expression is significantly associated with chemotherapy resistance and adverse prognosis in non-serous epithelial ovarian cancer patients." J Exp Clin Cancer Res **36**(1): 63.

Teh, M. T., E. Gemenetzidis, T. Chaplin, B. D. Young and M. P. Philpott (2010). "Upregulation of FOXM1 induces genomic instability in human epidermal keratinocytes." Mol Cancer **9**: 45.

Ter Brugge, P., P. Kristel, E. van der Burg, U. Boon, M. de Maaker, E. Lips, L. Mulder, J. de Ruiter, C. Moutinho, H. Gevensleben, E. Marangoni, I. Majewski, K. Jozwiak, W. Kloosterman, M. van Roosmalen, K. Duran, F. Hogervorst, N. Turner, M. Esteller, E. Cuppen, J. Wesseling and J. Jonkers (2016). "Mechanisms of Therapy Resistance in Patient-Derived Xenograft Models of BRCA1-Deficient Breast Cancer." J Natl Cancer Inst **108**(11).

Trimbos, J. B. (2017). "Surgical treatment of early-stage ovarian cancer." Best Pract Res Clin Obstet Gynaecol **41**: 60-70.

Tripathi, S., M. O. Pohl, Y. Zhou, A. Rodriguez-Frandsen, G. Wang, D. A. Stein, H. M. Moulton, P. DeJesus, J. Che, L. C. Mulder, E. Yanguez, D. Andenmatten, L. Pache, B. Manicassamy, R. A. Albrecht, M. G. Gonzalez, Q. Nguyen, A. Brass, S. Elledge, M. White, S. Shapira, N. Hacohen, A. Karlas, T. F. Meyer, M. Shales, A. Gatorano, J. R. Johnson, G. Jang, T.

Johnson, E. Verschueren, D. Sanders, N. Krogan, M. Shaw, R. Konig, S. Stertz, A. Garcia-Sastre and S. K. Chanda (2015). "Meta- and Orthogonal Integration of Influenza "OMICs" Data Defines a Role for UBR4 in Virus Budding." Cell Host Microbe **18**(6): 723-735.

Trope, C. and J. Kaern (2006). "Primary surgery for ovarian cancer." Eur J Surg Oncol **32**(8): 844-852.

Tsai, J., J. T. Lee, W. Wang, J. Zhang, H. Cho, S. Mamo, R. Bremer, S. Gillette, J. Kong, N. K. Haass, K. Sproesser, L. Li, K. S. Smalley, D. Fong, Y. L. Zhu, A. Marimuthu, H. Nguyen, B. Lam, J. Liu, I. Cheung, J. Rice, Y. Suzuki, C. Luu, C. Settachatgul, R. Shellooe, J. Cantwell, S. H. Kim, J. Schlessinger, K. Y. Zhang, B. L. West, B. Powell, G. Habets, C. Zhang, P. N. Ibrahim, P. Hirth, D. R. Artis, M. Herlyn and G. Bollag (2008). "Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity." Proc Natl Acad Sci U S A **105**(8): 3041-3046.

Turner, N., A. Tutt and A. Ashworth (2004). "Hallmarks of 'BRCAness' in sporadic cancers." Nat Rev Cancer **4**(10): 814-819.

Turner, N. C., C. J. Lord, E. Iorns, R. Brough, S. Swift, R. Elliott, S. Rayter, A. N. Tutt and A. Ashworth (2008). "A synthetic lethal siRNA screen identifying genes mediating sensitivity to a PARP inhibitor." EMBO J **27**(9): 1368-1377.

Tutt, A., M. Robson, J. E. Garber, S. M. Domchek, M. W. Audeh, J. N. Weitzel, M. Friedlander, B. Arun, N. Loman, R. K. Schmutzler, A. Wardley, G. Mitchell, H. Earl, M. Wickens and J. Carmichael (2010). "Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial." Lancet **376**(9737): 235-244.

Vaidyanathan, A., L. Sawers, A. L. Gannon, P. Chakravarty, A. L. Scott, S. E. Bray, M. J. Ferguson and G. Smith (2016). "ABCB1 (MDR1) induction defines a common resistance mechanism in paclitaxel- and olaparib-resistant ovarian cancer cells." Br J Cancer **115**(4): 431-441.

van Gent, D. C., J. H. Hoeijmakers and R. Kanaar (2001). "Chromosomal stability and the DNA double-stranded break connection." Nat Rev Genet **2**(3): 196-206.

van Oosterom, A. T., I. Judson, J. Verweij, S. Stroobants, E. Donato di Paola, S. Dimitrijevic, M. Martens, A. Webb, R. Sciot, M. Van Glabbeke, S. Silberman, O. S. Nielsen, R. European Organisation for, T. Treatment of Cancer Soft and G. Bone Sarcoma (2001). "Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study." Lancet **358**(9291): 1421-1423.

Vaughan, S., J. I. Coward, R. C. Bast, Jr., A. Berchuck, J. S. Berek, J. D. Brenton, G. Coukos, C. C. Crum, R. Drapkin, D. Etemadmoghadam, M. Friedlander, H. Gabra, S. B. Kaye, C. J. Lord, E. Lengyel, D. A. Levine, I. A. McNeish, U. Menon, G. B. Mills, K. P. Nephew, A. M. Oza, A. K. Sood, E. A. Stronach, H. Walczak, D. D. Bowtell and F. R. Balkwill (2011). "Rethinking ovarian cancer: recommendations for improving outcomes." Nat Rev Cancer **11**(10): 719-725.

Veeck, J., S. Ropero, F. Setien, E. Gonzalez-Suarez, A. Osorio, J. Benitez, J. G. Herman and M. Esteller (2010). "BRCA1 CpG island hypermethylation predicts sensitivity to poly(adenosine diphosphate)-ribose polymerase inhibitors." J Clin Oncol **28**(29): e563-564; author reply e565-566.

Vichai, V. and K. Kirtikara (2006). "Sulforhodamine B colorimetric assay for cytotoxicity screening." Nat Protoc **1**(3): 1112-1116.

Visintin, I., Z. Feng, G. Longton, D. C. Ward, A. B. Alvero, Y. Lai, J. Tenthorey, A. Leiser, R. Flores-Saaib, H. Yu, M. Azori, T. Rutherford, P. E. Schwartz and G. Mor (2008). "Diagnostic markers for early detection of ovarian cancer." Clin Cancer Res **14**(4): 1065-1072.

Wang, D., C. Li, Y. Zhang, M. Wang, N. Jiang, L. Xiang, T. Li, T. M. Roberts, J. J. Zhao, H. Cheng and P. Liu (2016). "Combined inhibition of PI3K and PARP is effective in the treatment of ovarian cancer cells with wild-type PIK3CA genes." Gynecol Oncol **142**(3): 548-556.

Wang, D., M. Wang, N. Jiang, Y. Zhang, X. Bian, X. Wang, T. M. Roberts, J. J. Zhao, P. Liu and H. Cheng (2016). "Effective use of PI3K inhibitor BKM120 and PARP inhibitor Olaparib to treat PIK3CA mutant ovarian cancer." Oncotarget **7**(11): 13153-13166.

Wang, M. and A. L. Gartel (2011). "Micelle-encapsulated thiostrepton as an effective nanomedicine for inhibiting tumor growth and for suppressing FOXM1 in human xenografts." Mol Cancer Ther **10**(12): 2287-2297.

Wang, M., W. Wu, W. Wu, B. Rosidi, L. Zhang, H. Wang and G. Iliakis (2006). "PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways." Nucleic Acids Res **34**(21): 6170-6182.

Wang, T., J. J. Wei, D. M. Sabatini and E. S. Lander (2014). "Genetic screens in human cells using the CRISPR-Cas9 system." Science **343**(6166): 80-84.

Wang, V., C. Li, M. Lin, W. Welch, D. Bell, Y. F. Wong, R. Berkowitz, S. C. Mok and C. A. Bandera (2005). "Ovarian cancer is a heterogeneous disease." Cancer Genet Cytogenet **161**(2): 170-173.

Wang, Y., L. Wen, S. H. Zhao, Z. H. Ai, J. Z. Guo and W. C. Liu (2013). "FoxM1 expression is significantly associated with cisplatin-based chemotherapy resistance and poor prognosis in advanced non-small cell lung cancer patients." Lung Cancer **79**(2): 173-179.

Wang, Z., S. Banerjee, D. Kong, Y. Li and F. H. Sarkar (2007). "Down-regulation of Forkhead Box M1 transcription factor leads to the inhibition of invasion and angiogenesis of pancreatic cancer cells." Cancer Res **67**(17): 8293-8300.

Wanka, C., J. P. Steinbach and J. Rieger (2012). "Tp53-induced glycolysis and apoptosis regulator (TIGAR) protects glioma cells from starvation-induced cell death by up-regulating respiration and improving cellular redox homeostasis." J Biol Chem **287**(40): 33436-33446.

Weber, S., C. K. McCann, D. M. Boruta, J. O. Schorge and W. B. Growdon (2011). "Laparoscopic surgical staging of early ovarian cancer." Rev Obstet Gynecol **4**(3-4): 117-122.

Weiswald, L. B., D. Bellet and V. Dangles-Marie (2015). "Spherical cancer models in tumor biology." Neoplasia **17**(1): 1-15.

Wilder, J. L., E. Pavlik, J. M. Straughn, T. Kirby, R. V. Higgins, P. D. DePriest, F. R. Ueland, R. J. Kryscio, R. J. Whitley and J. Nagell (2003). "Clinical implications of a rising serum CA-125 within the normal range in patients with epithelial ovarian cancer: a preliminary investigation." Gynecol Oncol **89**(2): 233-235.

Wurzer, G., Z. Herceg and J. Wesierska-Gadek (2000). "Increased resistance to anticancer therapy of mouse cells lacking the poly(ADP-ribose) polymerase attributable to up-regulation of the multidrug resistance gene product P-glycoprotein." Cancer Res **60**(15): 4238-4244.

Yang, D., S. Khan, Y. Sun, K. Hess, I. Shmulevich, A. K. Sood and W. Zhang (2011). "Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer." JAMA **306**(14): 1557-1565.

Yang, L., Y. Zhang, W. Shan, Z. Hu, J. Yuan, J. Pi, Y. Wang, L. Fan, Z. Tang, C. Li, X. Hu, J. L. Tanyi, Y. Fan, Q. Huang, K. Montone, C. V. Dang and L. Zhang (2017). "Repression of BET activity sensitizes homologous recombination-proficient cancers to PARP inhibition." Sci Transl Med **9**(400).

Yap, T. A., A. Omlin and J. S. de Bono (2013). "Development of therapeutic combinations targeting major cancer signaling pathways." J Clin Oncol **31**(12): 1592-1605.

Yap, T. A., S. K. Sandhu, C. P. Carden and J. S. de Bono (2011). "Poly(ADP-ribose) polymerase (PARP) inhibitors: Exploiting a synthetic lethal strategy in the clinic." CA Cancer J Clin **61**(1): 31-49.

Yardley, D. A. (2013). "Drug resistance and the role of combination chemotherapy in improving patient outcomes." Int J Breast Cancer **2013**: 137414.

Yin, L., M. Kosugi and D. Kufe (2012). "Inhibition of the MUC1-C oncoprotein induces multiple myeloma cell death by down-regulating TIGAR expression and depleting NADPH." Blood **119**(3): 810-816.

Yin, L., T. Kufe, D. Avigan and D. Kufe (2014). "Targeting MUC1-C is synergistic with bortezomib in downregulating TIGAR and inducing ROS-mediated myeloma cell death." Blood **123**(19): 2997-3006.

Ying, S., F. C. Hamdy and T. Helleday (2012). "Mre11-dependent degradation of stalled DNA replication forks is prevented by BRCA2 and PARP1." Cancer Res **72**(11): 2814-2821.

Yoshida, K. and Y. Miki (2004). "Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage." Cancer Sci **95**(11): 866-871.

Yu, H. P., J. M. Xie, B. Li, Y. H. Sun, Q. G. Gao, Z. H. Ding, H. R. Wu and Z. H. Qin (2015). "TIGAR regulates DNA damage and repair through pentosephosphate pathway and Cdk5-ATM pathway." Sci Rep **5**: 9853.

Zack, T. I., S. E. Schumacher, S. L. Carter, A. D. Cherniack, G. Saksena, B. Tabak, M. S. Lawrence, C. Z. Zhsng, J. Wala, C. H. Mermel, C. Sougnez, S. B. Gabriel, B. Hernandez, H. Shen, P. W. Laird, G. Getz, M. Meyerson and R. Beroukhim (2013). "Pan-cancer patterns of somatic copy number alteration." Nat Genet **45**(10): 1134-1140.

Zaidi, S., M. McLaughlin, S. A. Bhide, S. A. Eccles, P. Workman, C. M. Nutting, R. A. Huddart and K. J. Harrington (2012). "The HSP90 inhibitor NVP-AUY922 radiosensitizes by abrogation of homologous recombination resulting in mitotic entry with unresolved DNA damage." PLoS One **7**(4): e35436.

Zander, S. A., A. Kersbergen, E. van der Burg, N. de Water, O. van Tellingen, S. Gunnarsdottir, J. E. Jaspers, M. Pajic, A. O. Nygren, J. Jonkers, P. Borst and S. Rottenberg (2010). "Sensitivity and acquired resistance of BRCA1;p53-deficient mouse mammary tumors to the topoisomerase I inhibitor topotecan." Cancer Res **70**(4): 1700-1710.

Zanoni, M., F. Piccinini, C. Arienti, A. Zamagni, S. Santi, R. Polico, A. Bevilacqua and A. Tesei (2016). "3D tumor spheroid models for in vitro therapeutic screening: a systematic approach to enhance the biological relevance of data obtained." Sci Rep **6**: 19103.

Zhang, J., P. L. Yang and N. S. Gray (2009). "Targeting cancer with small molecule kinase inhibitors." Nat Rev Cancer **9**(1): 28-39.

Zhang, N., X. Wu, L. Yang, F. Xiao, H. Zhang, A. Zhou, Z. Huang and S. Huang (2012). "FoxM1 inhibition sensitizes resistant glioblastoma cells to temozolomide by downregulating the expression of DNA-repair gene Rad51." Clin Cancer Res **18**(21): 5961-5971.

Zhang, X., L. Cheng, K. Minn, R. Madan, A. K. Godwin, V. Shridhar and J. Chien (2014). "Targeting of mutant p53-induced FoxM1 with thiostrepton induces cytotoxicity and enhances carboplatin sensitivity in cancer cells." Oncotarget **5**(22): 11365-11380.

Zhang, Z., P. Ma, Y. Jing, Y. Yan, M. C. Cai, M. Zhang, S. Zhang, H. Peng, Z. L. Ji, W. Di, Z. Gu, W. Q. Gao and G. Zhuang (2016). "BET Bromodomain Inhibition as a Therapeutic Strategy in Ovarian Cancer by Downregulating FoxM1." Theranostics **6**(2): 219-230.

Zhao, F., M. K. Siu, L. Jiang, K. F. Tam, H. Y. Ngan, X. F. Le, O. G. Wong, E. S. Wong, A. R. Gomes, L. Bella, P. Khongkow, E. W. Lam and A. N. Cheung (2014). "Overexpression of forkhead box protein M1 (FOXM1) in ovarian cancer correlates with poor patient survival and contributes to paclitaxel resistance." PLoS One **9**(11): e113478.

Zhou, B., J. Hu, F. Xu, Z. Chen, L. Bai, E. Fernandez-Salas, M. Lin, L. Liu, C. Y. Yang, Y. Zhao, D. McEachern, S. Przybranowski, B. Wen, D. Sun and S. Wang (2017). "Discovery of a Small-Molecule Degrader of Bromodomain and Extra-Terminal (BET) Proteins with Picomolar Cellular Potencies and Capable of Achieving Tumor Regression." J Med Chem.

Zona, S., L. Bella, M. J. Burton, G. Nestal de Moraes and E. W. Lam (2014). "FOXM1: an emerging master regulator of DNA damage response and genotoxic agent resistance." Biochim Biophys Acta **1839**(11): 1316-1322.

Zuber, J., J. Shi, E. Wang, A. R. Rappaport, H. Herrmann, E. A. Sison, D. Magoon, J. Qi, K. Blatt, M. Wunderlich, M. J. Taylor, C. Johns, A. Chicas, J. C. Mulloy, S. C. Kogan, P. Brown, P. Valent, J. E. Bradner, S. W. Lowe and C. R. Vakoc (2011). "RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia." Nature **478**(7370): 524-528.