Hydrating for Resistance to Radicicol

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Abstract

Resistance to Hsp90 inhibition has become an important concern as several clinical trials are currently in progress for the treatment of cancer. A summary of known mechanisms of resistance to Hsp90 inhibitors is provided, including the recent solution of the Humicola fuscoatra Hsp90 structure, the organism responsible for the biosynthesis of radicicol. Through careful analyses of Hsp90 structures, a plausible mechanism for resistance to Hsp90 inhibitors has been obtained by single mutations about the N-terminal ATP-binding site.

The 90-kDa heat shock proteins (Hsp90) represent a highly conserved class of ATP-dependent molecular chaperones responsible for the maturation of newly formed polypeptides and the stability and/or rematuration of denatured polypeptides (1). Although Hsp90 is essential for normal cell development and proliferation, research has also shown its clientele to include a myriad of oncogenic proteins. Furthermore, Hsp90-dependent substrates are directly associated with all six hallmarks of cancer, allowing Hsp90 inhibition to simultaneously disrupt all oncogenic traits (2).

Known inhibitors act by binding to the N-terminal ATP-binding site, and the potential for evolving drug resistance via mutation of this domain has been dismissed in both scientific discussions as well as the literature. The dominant opinion has been that mutations to the ATP-binding pocket would render Hsp90 unable to bind/hydrolyze ATP and would therefore compromise its chaperone function. Consequently, resistance to Hsp90 inhibition was believed limited to drug efflux or metabolic mechanisms of drug inactivation. In contrast to this opinion, Prodomou et al. (DOI 10.1021/cb9000316) (3) have performed site-directed mutagenesis to demonstrate that mutation within the Hsp90 N-terminal ATP-binding pocket results in selective resistance to radicicol (RDC, Figure 1), while maintaining susceptibility to inhibition by geldanamycin (GDA) and sustaining ATP-hydrolysis activity.

The ability of Hsp90 inhibitors to affect multiple oncogenic signaling pathways has propelled their potential use for the treatment of various cancers. However, resistance to Hsp90 inhibitors has been established and liabilities should be considered when developing new therapeutics. The first example of resistance to Hsp90 inhibitors was discovered prior to identification of the chaperone as the biological target of the ansamycin, GDA. Research by Benchekroun et al. (4) noted the ability of cancer cells to acquire resistance to the ansamycin class of natural products, which was believed to be multifactorial. Subsequent disclosure of Hsp90 as the target for GDA and recent confirmation of Hsp90 as a viable anticancer target have inspired further research aimed at elucidating the mechanisms of resistance to Hsp90 inhibitors.

One of the first reports of acquired resistance to Hsp90 inhibitors was noted in hormone-refractory breast cancer cells with a semisynthetic analog of GDA, 17-AAG (5). An increase

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in drug efflux and metabolic alterations were cited as the basis for resistance. Accordingly, Benchekroun and colleagues used photoaffinity-labeling experiments to show that GDA interacts with the P-170 glycoprotein drug efflux pump (P-gp) and that resistant cells expressing this efflux pump exhibited decreased intracellular GDA concentrations (Figure 2, panel a). Additional work has confirmed that GDA is both a substrate and an inhibitor of P-gp. Not surprisingly, studies have demonstrated that P-gp inhibition resensitizes cells to GDA, and recent studies by McCollum et al. (6) have reiterated the role of drug efflux pumps for exhibiting resistance to Hsp90 inhibitors. In addition, they have also demonstrated induction of the stress response as an additional factor in the development of resistance (Figure 2, panel b). For example, when GDA-resistant A549 cells were transfected with Hsp27 and/or Hsp70 siRNA, sensistivity to GDA increased 10-fold, whereas pretreatment with the P-gp inhibitor verapamil did not resensitize these cells.

Another hypothesis put forth by Benchekroun’s report was that resistance developed from alteration of the cellular reduction potential (Figure 2, panel c). Subsequent studies identified NAD(P)H/quinone oxidoreductase 1 (NQ01) as the enzyme responsible for reducing the quinone ring of GDA to the higher affinity (~40-fold), hydroquinone. Studies by Gaspar et al. (7) indicated an inverse correlation between NQ01 expression and the IC$_{50}$ of 17-AAG, suggesting the mechanism of resistance is a direct consequence of reduction of NQ01 activity. Although some cell lines maintain lower NQ01 activity, results obtained from Gasper and colleagues provided the first example of acquired resistance via this mechanism.

Recent elucidation of Hsp90 cochaperones has led to studies aimed at determining the function of these assistants, as a number of cochaperones are intimately involved with Hsp90 function (8). For instance, Cox and Miller (9) monitored downstream signaling effects produced by the aryl hydrocarbon receptor (AhR), an Hsp90-dependent transcription factor, to probe the relationship between p23/Sba1 and Hsp90 inhibitors (Figure 2, panel d). Their work not only indicated p23/Sba1 as an N-terminal interactor, but also elucidated its role as a putative mechanism of resistance to Hsp90 inhibition. Cox and Miller showed that overexpression of p23/Sba1 diminished the inhibitory activity of N-terminal inhibitors, namely radicicol and herbimycin A. In separate studies, Forafonov et al. (10) demonstrated that in the absence of p23/Sba1 yeast and mammalian cells were more susceptible to Hsp90 inhibition. The competitive role of p23/Sba1 results from their function to bind and stabilize the Hsp90 ATP-bound form, thus blocking hydrolysis and preventing inhibitor binding. Evidence has shown that mutants of p23/Sba1 are viable (11), suggesting that p23/Sba1 may fortuitously represent the first evolutionary mechanism to protect cells from Hsp90 inhibition. Now, with the work of Prodromou and colleagues, there is evidence that further evolutionary pressures may result in Hsp90 mutants that are capable of hydrolyzing ATP and carrying out normal cellular functions without the potential of inhibition.

Fungal secondary metabolites have played an important role in medicinal chemistry, especially in the development of both antimicrobial and antitumor chemotherapies (12). RDC is isolated from Humicola fuscoatra (13) and has been shown to be a potent inhibitor of Hsp90 in vitro. Not surprisingly, Hsp90 in H. fuscoatra exhibits much lower affinity for RDC than yeast or mammalian homologues because this organism must protect itself from its own biosynthetic products. Isolation and sequencing of the Hsp90 gene from H. fuscoatra revealed two minor differences in the ATP-binding pocket when compared with that of yeast Hsp90. Therefore, the authors sought to incorporate these two mutations into yeast Hsp90, which is normally susceptible to RDC inhibition. Using site-directed mutagenesis, the authors were able to show that mutation of leucine to isoleucine resulted in selective resistance to RDC by yeast Hsp90. When the yeast L34I mutant Hsp90 was constructed, resistance to radicicol was observed, clearly indicating that a single point mutation is sufficient to cause resistance in other
organisms. Surprisingly, sensitivity to GDA was unaffected and ATP hydrolysis proceeded at an unaffected rate.

To investigate the diminished RDC inhibitory activity that did not reduce GDA or ATP affinity, Prodromou et al. solved the co-crystal structures of the three ligands bound to mutant Hsp90. Comparative analyses of the structures revealed that the L34I mutation altered the hydration state within the binding pocket by allowing the incorporation of three additional water molecules, resulting in an unfavorable environment for the chlorine substituent of RDC. The change in hydration state, however, did not affect ATP binding due to the absence of hydrophobic groups on the adenine ring. In the case of GDA, the molecule is too large and does not allow for the accommodation of these water molecules, causing GDA’s affinity to be unaffected as these water molecules are simply displaced upon binding of the natural product. In a conclusive study, Prodromou and co-workers demonstrated that minor perturbation of the Hsp90 ATP-binding pocket can significantly alter the affinity for specific inhibitory ligands (Figure 2, panel e).

The studies by Prodromou and colleagues indicate that Hsp90 can retain its chaperone function despite mutations to the ATP-binding pocket. Although these studies provide evolutionary evidence for a mechanism of resistance to Hsp90 inhibition by RDC, research will demonstrate whether pressures can produce such mutations in mammalian cells. In addition, this work begs the question as to how *Streptomyces hygroscopicus*, the bacteria responsible for GDA biosynthesis, is protected from Hsp90 inhibition, as GDA analogues are further advanced in clinical trials than any other Hsp90 inhibitor. Therefore, these studies reemphasize the importance of understanding the interaction of ligands with Hsp90 and reaffirm the need for new chemical scaffolds that inhibit the Hsp90 protein folding machinery.

References


Figure 1.
Hsp90 N-terminal ligands.
Figure 2.
Mechanisms of resistance to Hsp90 inhibitors: a) drug efflux via P-gp pumps; b) induction of heat shock response; c) decreased NQ01 enzymatic activity, which is responsible for reduction of the GDA quinone to the higher affinity hydroquinone; d) competitive binding of p23/Sba1 to the Hsp90 N-terminus; and e) mutation to the N-terminal binding pocket (L34I), which alters the hydration state.