DEVELOPMENT OF NUCLEAR RECEPTOR TRANSFECTED CACO-2 CELL LINES

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• **Background**
  
• Cell lines

• Gene expression

• Functional experiments

• Conclusions
Intestinal absorption

• Small intestine is the most important site of drug absorption ➔ predictions in the discovery phase important
• Intestinal epithelium often limits the absorption rate from GIT
• Active first-pass metabolism
CYPs and efflux pumps

- Cytochrome P450 (CYP) families 1-3 metabolise several xenobiotic compounds in microsomes of several tissues (e.g. liver, small intestine)

- Efflux-pumps (P-glycoprotein, MRP-family, BCRP) excrete several xenobiotic compounds from the cells

CYPs / efflux pumps - interplay

- = CYP enzyme
- = Efflux pump
- = Drug molecule
- OH = oxidised drug molecule
Regulation of xenobiotic metabolism - nuclear receptors

Some target genes:
- CYP2B6
- CYP2C9
- CYP3A4
- MDR1

Honkakoski, Current Pharmacogenomics 2003(1):75-85
Average enterocyte?

• Background
• **Cell lines**
• Gene expression
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Caco-2 cells

A colon carcinoma cell line that differentiates spontaneously into enterocyte-like cells

+ Widely used
+ Human origin
+ Expresses many transporters
+ Relatively easy to grow
- Inter and intralaboratory differences
- Paracellular space very tight
- Incomplete transporter profile
- CYP metabolism absent
- Long growth time

About Caco-2 cells in permeability experiments:
# Modified cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Modification</th>
<th>Ligands</th>
<th>Some target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caco/WT</td>
<td>Wild type cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caco/hPXR</td>
<td>Transfection with human PXR</td>
<td>+: Rifampicin, ritonavir,</td>
<td><strong>CYP3A4, MDR1, CYP2B6</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>hyperforin</td>
<td></td>
</tr>
<tr>
<td>Caco/mCAR</td>
<td>Transfection with murine CAR</td>
<td>+: TCPOBOP,</td>
<td>**CYP2B6, MDR1, CYP3A4, CYP2C9, MRP2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenobarbital</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-: Androstenol, progesterone</td>
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</tbody>
</table>

Initial characterisation:

Induction properties:
• Background
• Cell lines
• **Gene expression**
• Functional experiments
• Conclusions
Changes in transcription: qRT-PCR

R = hPXR activator rifampicin, T = mCAR activator TCPOBOP, A = mCAR inhibitor androstenol

CYP3A4

CYP2B6

<table>
<thead>
<tr>
<th>Rifampicin (days)</th>
<th>Vehicle</th>
<th>14</th>
<th>7</th>
<th>3</th>
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<tbody>
<tr>
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<td>40</td>
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<tbody>
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<td>2</td>
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<table>
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MDR1

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</table>
## Protein level

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Caco/WT</th>
<th>Caco/hPXR</th>
<th>Caco/mCAR</th>
<th>Caco/VD3</th>
<th>CYP3A4 (fmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>-</td>
<td>- Rifampicin</td>
<td>- TCPOBOP Androstenol</td>
<td>MDCK-MDR1</td>
<td>100  50  10</td>
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</tbody>
</table>

**CYP3A4**

**P-gp**
- Background
- Cell lines
- Gene expression
- **Functional experiments**
- Conclusions
Permeability experiments

- Apical to basolateral (AB): "from intestinal lumen to circulation"
- Basolateral to apical (BA): "from circulation to intestinal lumen"
- $P_{AB} > P_{BA}$ -> Active absorption
- $P_{AB} < P_{BA}$ -> Efflux pump (active secretion)
- pH-gradients may cause deviations if ionisable molecules are studied!
Passive permeability

Antipyrine (transcellular)

Mannitol (paracellular)
Active absorption

Cephalexin (hPepT1)

- $P_{app} \times 10^6$
Permeability involving P-gp

- AB
- AB+inhibitor
- BA+inhibitor
- BA

Apparent permeability (x 10^6 cm/s)

Cell line: Caco/WT, Caco/hPXR, Caco/mCAR
Treatment: - , Rifampicin, - , TCPOBOP, Androstenedol

Digoxin
Permeability involving P-gp

Quinidine

- AB
- AB+inhibitor
- BA+inhibitor
- BA

Apparent permeability (x 10^6 cm/s)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Caco/WT</th>
<th>Caco/hPXR</th>
<th>Caco/mCAR</th>
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</thead>
<tbody>
<tr>
<td>AB</td>
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<tr>
<td>AB+inhibitor</td>
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<td></td>
</tr>
<tr>
<td>BA+inhibitor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA</td>
<td></td>
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</table>
CYP3A4 mediated metabolism

Ketoconazole sensitive HFC formation from BFC

![Graph showing HFC concentration (nM) for different cell lines and treatments.](image-url)
Conclusions

• Stable transfectants retain viability and passive transport properties
• Genes can be controlled in Caco-2 cells with nuclear receptors and their ligands
• P-gp induction can also be seen at protein and functional levels
• CYP3A4 protein level and activity remain very modest
Acknowledgements

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