A Microarray for ABC Transporter Gene Expression Profiling

Rob Shipman, Erin Rose, Katherine Marseu, Joti Sidhu and David K.H. Lee, NoAb BioDiscoveries Inc., Mississauga, Ontario CANADA

NoAb BioDiscoveries

Purpose

To investigate the basal gene expression levels and drug associated changes in gene expression levels of the 48 known human ABC transporters (ABCTs) in cell lines using a human ABC transporter microarray.

Methods

ABCT gene expression was determined by extraction of total RNA from cells lines cultured to ~80% confluency in the presence or absence of drug treatment. Total RNA was labelled with Cy3-random nonamers, column-purified and hybridised to denatured, blocked and pre-hybridised ABCT microarrays for 18 hours at 37°C. Microarrays were washed in 0.1xSSC for 1hour at 37°C, dipped in de-ionised water and spin-dried at 1200rpm for 5 minutes at room temperature.
Microarray image acquisition was performed in a GSI Lumonics ScanArray Lite (Figure 1). Image analysis was performed using Quantarray. Matrix plots were generated using GeneLinker Gold. Histograms were prepared using Microsoft Office Excel.

Results

1. Basal levels of ABCT gene expression differ between cell lines. Most naïve cell lines express multiple ABCTs at levels at least 2 times that of control housekeeping genes (Figure 2).
2. Different levels and patterns of ABCT gene expression are induced by different drugs in cell lines of different tissue origin (Figure 3 & Figure 4).
3. ABCT gene expression peaks at 8h for vinblastine treated HepG2 cells and at 4h for A549 cells. ABCT gene expression peaks at 2h for doxorubicin treated HepG2 cells and at 8h for A549 cells. Both drugs induce ABCT gene expression to levels at least 5 times that of control housekeeping genes and 2-fold over the untreated control (Figure 3).
4. Preferential induction of a number of ABCTs by doxorubicin and vinblastine in both A549 and HepG2 cell lines was also observed (Figures 3).
5. NSAIDs generate different patterns of ABCT gene expression in different cell lines (Figure 4).

Conclusions

1. ABCT microarrays can be used to assess changes in ABC transporter gene expression levels in drug-treated and naïve cell lines.
2. ABCT gene expression levels vary depending on the drug, drug concentration and duration of exposure to the drug.
3. Multiple ABCTs are involved in the response to drug treatment. This pattern of ABCT expression may represent an early dynamic response to xenobiotic disposition in these cells.

Caco-2

HEK293

Figure 1. ABCT microarray hybridisation images
Total RNA was isolated from naïve or drug-treated cell lines, labelled and hybridised to individual ABCT microarrays as described in the *Methods*.

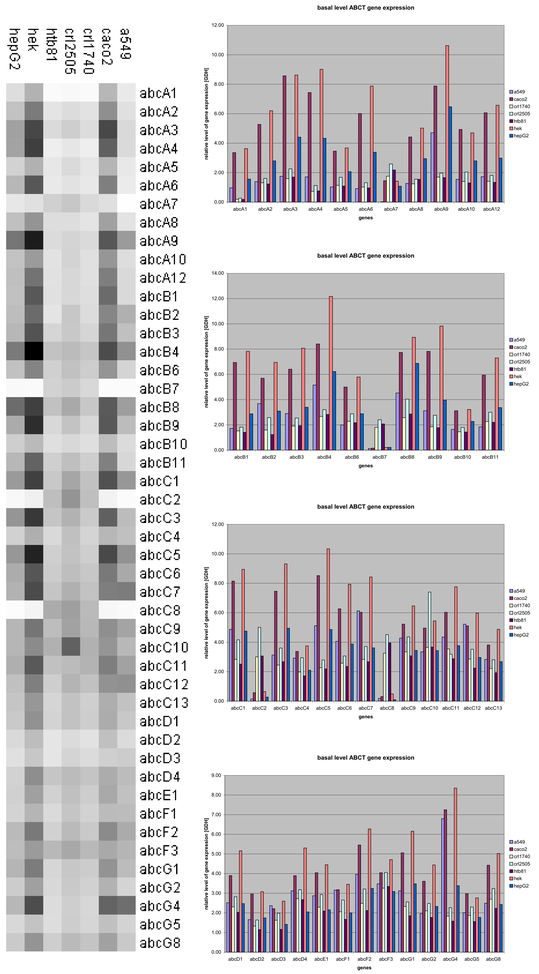

Figure 2. Basal ABCT gene expression levels in seven untreated cell lines

Total RNA from the A549 (lung), CaCo2 (intestine), CRL1740 (prostate), CRL2505 (prostate), HTB81 (prostate), HEK293 (kidney) and HepG2 (liver) cell lines was labelled and hybridised to individual ABCT microarrays as described in the *Methods*. Normalised, background subtracted fluorescence intensity values from each microarray hybridisation experiment were used to generate both the matrix plot and histogram.

Figure 3. ABCT gene expression levels in doxorubicin and vinblastine treated A549 and HepG2 cell lines.

Total RNA from A549 and HepG2 cells treated with doxorubicin (50nM) or vinblastine (50nM) for 0, 2, 4 and 8h was labelled and hybridised to individual ABCT microarrays as described in the *Methods*. Normalised, background subtracted fluorescence intensity values from each microarray hybridisation experiment were used to generate both the matrix plot and histogram.

Figure 4. Fold-change in ABCT gene expression levels in NSAID (non-steroidal anti-inflammatory drug) treated cell lines.

Total RNA from cell lines treated with NSAIDs (acetaminophen [50uM] or acetylsalicylic acid [50uM]) for 48h was labelled and hybridised to individual ABCT microarrays as described in the *Methods*. Normalised, background subtracted fluorescence intensity values from each microarray hybridisation experiment were used to generate the primary data. Fold-change values represent a ratio of the normalised, background subtracted fluorescence intensity values of the NSAID-treated sample versus the untreated control sample for each cell line and NSAID combination. These values were used to generate both the matrix plot and histogram. **AAP** = acetaminophen, **ASA** = acetylsalicylic acid.