Gene Expression Profiling using a Human ABC Transporter Microarray

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Purpose

To assess the gene expression levels and profiles of the 48 known human ABC transporters (ABCTs) in naïve and treated 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 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 2). Image analysis was performed using Quantarray. Matrix plots were generated using GeneLinker Gold. Histograms were prepared using Microsoft Office Excel.

ABCT microarrays were printed from purified ABCT pcr products [~50ng/ul] in 150mM sodium phosphate pH8.5 on epoxy activated glass slides using an ESI SDDC-2 arrayer and TeleChem SMP5 pins.

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 1).

2. The levels of ABCT gene expression detected by microarray and semi-quantitative PCR are equivalent (Figures 3 and 4).

3. Different levels and patterns of ABCT gene expression are observed in cell lines treated with different compounds. (Figures 6, 7 and 8).

4. Methanol, phenobarbital, acetylsalicylic acid and acetaminophen induce [abcA9, B4 and G4] and suppress [abcB7, C2 and C8] ABCDT gene expression to various degrees in different prostate cancer cell lines (Figure 6).

5. Doxorubicin and vinblastine induce the expression of ABCT genes to different levels and with different kinetics in A549 cells [abcA3, B8, C1 and G1] (Figure 7).

6. Retinoids produce variable levels of ABCT gene expression in A549 cells. all-trans-retinoic acid (AAT) suppresses ABCT gene expression [abcC8 and C10] whereas 13-cis-retinoic acid (A13) induces ABCT expression [abcC1, D4, F3 and G8] (Figure 8).

Conclusions

1. ABCT microarrays can be used to assess the magnitude of ABC transporter gene expression in naïve cell lines and the changes in ABCT gene expression levels in treated cell lines.

2. The magnitude of ABCT gene expression in treated cell lines is dependent on the compound and duration of exposure to the compound.

3. Multiple ABCTs are involved in the response to treatment with various drugs or other compounds. The observed pattern of ABCT gene expression may represent an early dynamic response to xenobiotic disposition in these cells.

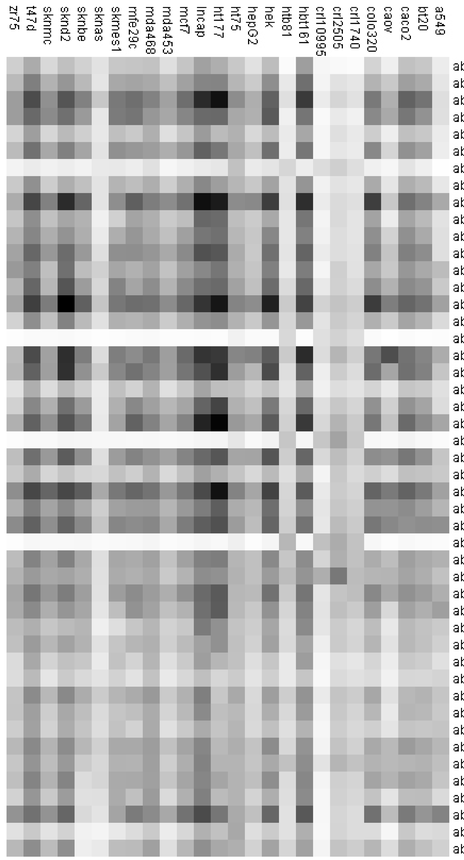


Figure 1. Basal ABCT gene expression levels in untreated cell lines
Total RNA [~20ug] from all 26 cell lines was labelled and hybridised to individual ABCT microarrays as described in the *Methods*. Normalised, background subtracted fluorescence intensity values from each microarray hybridisation experiment was used to generate the matrix plot.

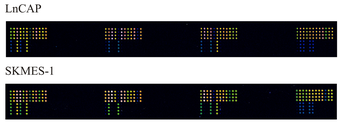


Figure 2. ABCT microarray hybridisation images
Total RNA was isolated from the cell lines, labelled and hybridised to individual ABCT microarrays as described in the *Methods*.

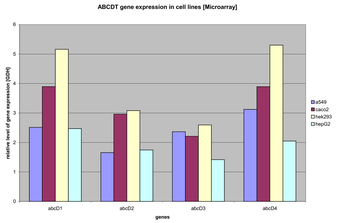


Figure 3. ABCD sub-family gene expression in four cell lines. The basal level of ABCD gene expression in A549 (lung), HEK (kidney), CaCo2 (intestine) and HepG2 (liver) cell lines was determined using the ABCDT microarray. Normalised, background subtracted fluorescence intensity values from the microarray hybridisation experiment were used to generate the histogram.

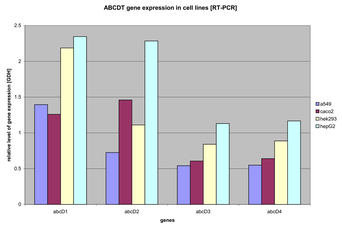


Figure 4. ABCD sub-family gene expression in four cell lines. RT-PCR was used to assess the basal level of ABCD gene expression in A549 (lung), HEK (kidney), CaCo2 (intestine) and HepG2 (liver) cell lines. Normalised, background subtracted band intensities were determined using a flatbed scanner and ImageJ software. The histogram was created from these values.

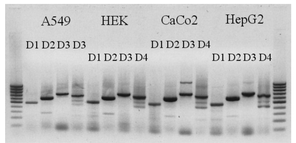
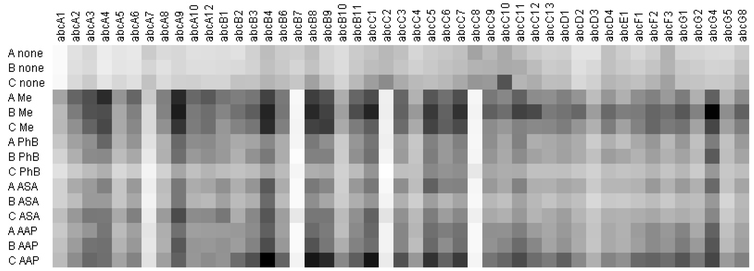


Figure 5. ABCD sub-family gene expression in four cell lines. RT-PCR was used to assess the basal level of ABCD gene expression in the A549 (lung), HEK (kidney), CaCo2 (intestine) and HepG2 (liver) cell lines.



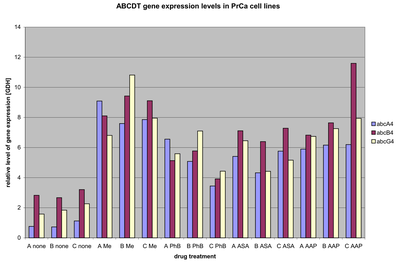
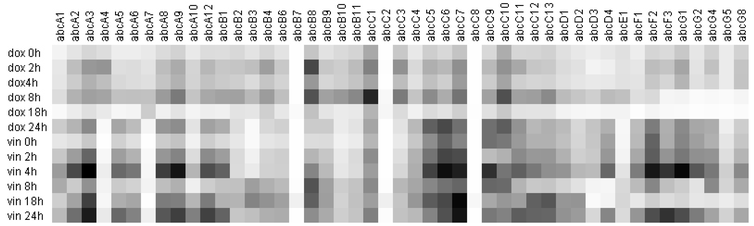


Figure 6. ABCT gene expression levels in three prostate cancer cell lines treated with various compounds

Total RNA [~20ug] from untreated and treated prostate cancer 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.
A = HTB81, B = CRL10995, C = CRL1740. Me: methanol [1%, 15h], PhB: phenobarbital [400uM, 48h], ASA: acetylsalicylic acid [50uM, 15h], AAP: acetaminophen [50uM, 15h]



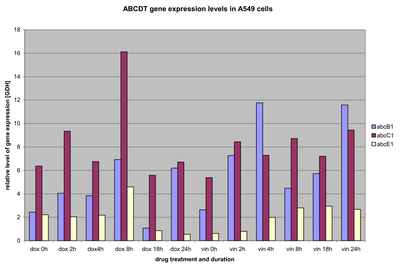
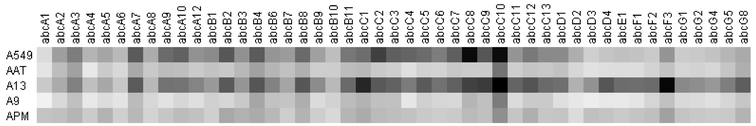


Figure 7. ABCT gene expression levels in A549 cells treated with doxorubicin or vinblastine

Total RNA [~20ug] from untreated and treated A549 cells 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. **dox** = doxorubicin [50nM for the indicated exposure periods], **vin** = vinblastine [50nM for the indicated exposure periods].



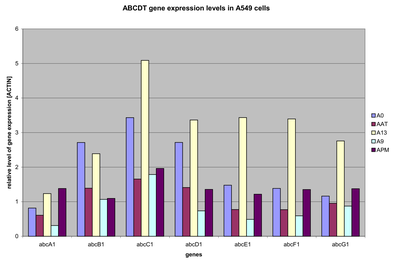


Figure 8. ABCT gene expression levels in retinoid treated A549 cells

Total RNA [~20ug] from untreated and retinoid treated A549 cells 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.
AAT: all-trans retinoic acid [10uM], A13: cis-13-retinoic acid [10uM], A9: cis-9-retinoic acid [10uM], APM: phorbol 12-myristate 13-acetate [10nM].