DTEx™ - Gene Expression Profiling using a Human ABC Transporter Microarray

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Introduction

ABC Transporters regulate the efflux of many intracellular metabolites and xenobiotic compounds. These"drug transporters" play a critical role in the disposition of administered drugs and have been implicated in multi-drug resistance in cancer chemotherapy.
Since different target organs are likely to express different numbers or levels of ABC transporters, identifying the pattern and magnitude of ABC transporter expression associated with drug treatment(s) may assist in predicting unusual observations or avoiding adverse events in clinical trials.

Purpose

1. To assess the gene expression levels of the ABC Transporters [ABCTs] in naïve and drug treated cell lines using the DTEx™ microarray.

2. To compare ABCT gene expression and ABCT protein expression levels in drug treated cell lines.

Methods

ABCT gene expression was determined by extracting total cellular RNA from cell lines cultured to ~80% confluency in the presence or absence of drugs using TriZol [Invitrogen]. Total RNA was labelled with either Cy5-random nonamers or the Message Amp™ II aRNA amplification kit [Ambion]. Labelled targets were hybridised to the pre-blocked DTEx™ microarray for 18 hours at 37°C. Microarrays were washed and spin-dried at 1200rpm for 5 minutes at room temperature. Microarray image acquisition was performed in a GSI Lumonics ScanArray Lite. Image analysis was performed using QuantArray. GeneLinker Gold [IOS] was used to generate the matrix plots.

DTEx™ microarrays were printed from purified ABCT vector pcr products [100ng/ul] in 150mM sodium phosphate pH8.5 onto 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 genes [Figure 1].

2. Different levels and patterns of ABCT gene expression are observed in doxorubicin treated cell lines [Figures 2, 3 and 4].
The level of ABCT gene expression varies with the period of exposure to doxorubicin.

3. The levels of ABCT gene expression detected by DTEx™ microarray analysis and semi - quantitative RT-PCR are equivalent [Figures 5, 6 and 7].

4. The levels of ABCT gene expression are comparable to the levels of ABCT protein expression in doxorubicin treated cells [Figure 8]. Increased ABCT gene expression correlates with increased ABCT protein expression which seems to represent "new" ABCT protein synthesis rather than release of pre-formed ABCT protein from intracellular pools [Figure 9].

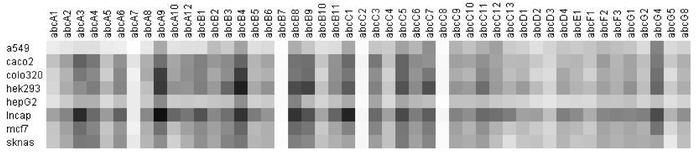

Figure 1. Basal ABCT gene expression in untreated human cell lines

Total RNA from all human cell lines was labelled and hybridised to individual DTEx™ microarrays as described in the Methods. Control gene normalised, background subtracted fluorescence intensity values from each microarray hybridisation experiment was use to generate the matrix plot.

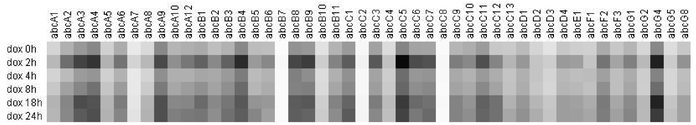

Figure 2. ABCT gene expression in doxorubicin treated HepG2 cells

Total RNA from doxorubicin treated [100nM for the indicated exposure periods] HepG2 cells was labelled and hybridised to individual DTEx™ microarrays as described in the Methods. Control gene normalised, background subtracted fluorescence intensity values from each microarray hybridisation experiment was use to generate the matrix plot.

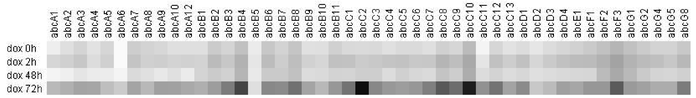

Figure 3. ABCT gene expression in doxorubicin treated HepG2 cells

Total RNA from doxorubicin treated [100nM for the indicated exposure periods] HepG2 cells was labelled and hybridised to individual DTEx™ microarrays as described in the Methods. Control gene normalised, background subtracted fluorescence intensity values from each microarray hybridisation experiment was use to generate the matrix plot.

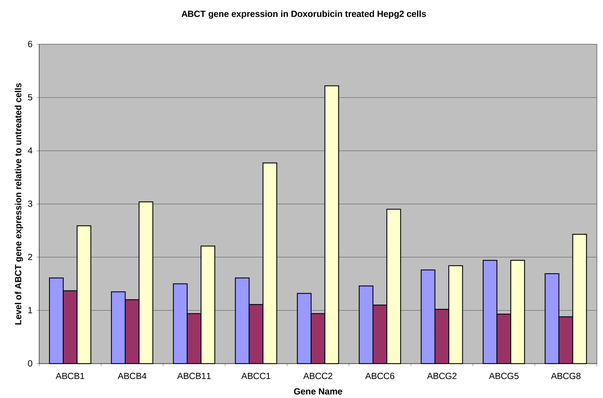

Figure 4. ABCT gene expression in doxorubicin treated HepG2 cells

Changes in the expression levels of selected ABCT genes were determined using DTEx™ microarrays. Control gene normalised, background subtracted fluorescence intensity values for untreated HepG2 cells and cells treated with doxorubicin for 24, 48 and 72 hours were used to generate the"fold-change" values shown in the histogram.

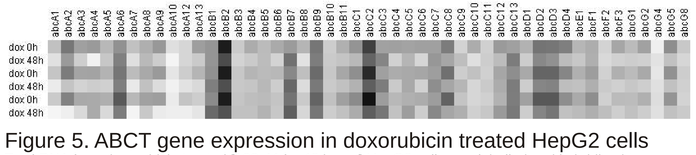

Figure 5. ABCT gene expression in doxorubicin treated HepG2 cells

Total RNA from doxorubicin treated [100nM for 48 hours] HepG2 cells was labelled and hybridised to individual DTEx™ microarrays as described in the Methods. Control gene normalised, background subtracted fluorescence intensity values from each microarray hybridisation experiment was use to generate the matrix plot.

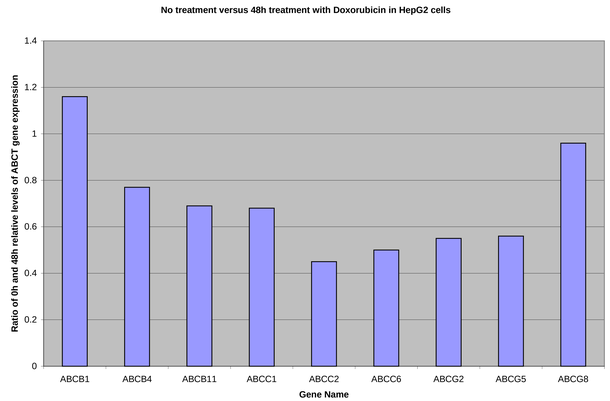

Figure 6. ABCT gene expression in doxorubicin treated HepG2 cells

Changes in the expression levels of selected ABCT genes were determined using DTEx™ microarrays. Control gene normalised, background subtracted fluorescence intensity values for untreated HepG2 cells and cells treated with doxorubicin for 48 hours were used to generate the"fold-change" values shown in the histogram.

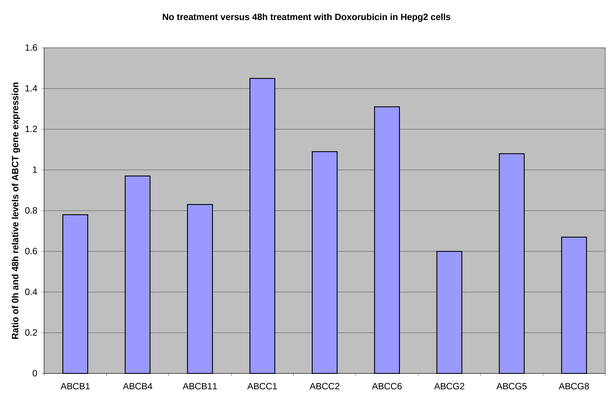

Figure 7. ABCT gene expression in doxorubicin treated HepG2 cells

Changes in the expression levels of selected ABCT genes were determined using RT-PCR. Control gene normalised, background subtracted fluorescence intensity values for untreated HepG2 cells and cells treated with doxorubicin for 48 hours were used to generate the"fold-change" values shown in the histogram.

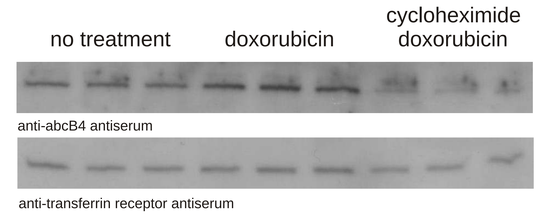

Figure 8. ABCB4 protein expression in doxorubicin treated A549 cells

A549 cell monolayers were treated with doxorubicin [100nM], cycloheximide [5ug/ml] plus doxorubicin [100nM] or no treatment for 48 hours.Cells were harvested and whole cell lysates were prepared. Membrane fractions were isolated using the Mem-PER kit [Pierce] according to the manufacturer's instructions. Protein concentrations were determined with the MicroBCA Protein Assay Reagent [Pierce]. Western blots onto PVDF membranes [Pall] from 25ug of each membrane sample were incubated with the indicated primary antibodies and developed with alk. phos. conjugated secondary antibodies and a chemiluminescent substrate [LumiPhos, BioRad]. Individual band intensities were determined by densitometric analysis of the X-OmatAR film using ImageJ software.

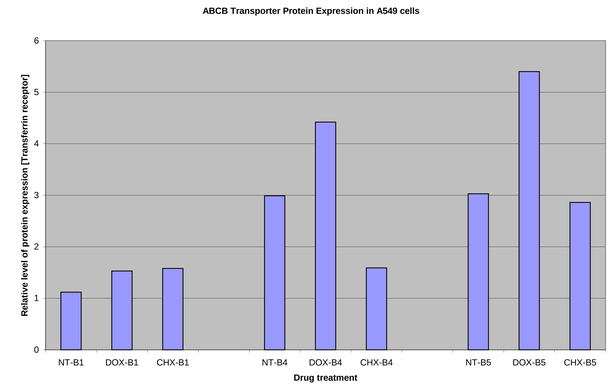

Figure 9. ABCB protein expression in doxorubicin treated A549 cells

A549 cell monolayers were treated with doxorubicin [100nM], cycloheximide [5ug/ml] plus doxorubicin [100nM] or no treatment for 48 hours. Western blots of membrane preparations from each of these samples were performed using anti-abcB1, anti-abcB4, anti-abcB5 and anti-transferrin receptor antibodies. Blots were developed and analysed as described for Figure 8.

Summary

1. DTEx™ microarrays can be used to assess changes in ABC transporter gene expression levels in naïve, untreated and drug-treated human cell lines.

2. ABC transporter gene expression levels vary with the duration of exposure to the drug.

3. Multiple ABC transporter genes are expressed in the response to drug treatment.

4. ABC transporter gene expression levels correlate with ABC transporter protein expression levels.

5. Doxorubicin induces both ABC transporter gene expression and "new" ABC transporter protein synthesis in cell lines.

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