Expression Analysis of ABC Transporters in Lung Cancer Cell Lines Using a RealTime ready qPCR Focus Panel

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Introduction

The novel small molecule inhibitors are often substrates of ABC transporters [1] which influence the pharmacokinetic parameters of the drug candidates and cause drug resistance of tumor cells [2,3]. Therefore expression profiling of ABC transporter genes in cell lines used for drug screening is highly important for anti-cancer drug discovery projects.

Our purpose was to create a basic expression profile of ABC transporters in our mainly used lung cancer cell lines using the RealTime ready ABC Transporter Panel. The RealTime ready ABC-Transporter Panel comprises the 42 most important human ABC transporters in duplicate. The panel includes five controls for RNA quality and seven reference gene assays. The assays are supplied in a LightCycler® Instrument 480 Multiwell Plate 96 and contain target specific primers and a FAM-labeled Universal ProbLibrary hydrolysis probe that contains LNA.

Materials and Methods

Cell lines

Cell lines were derived from ATCC database and maintained following database's recommendations (Table 1).

RNA isolation and cDNA preparation

RNA was isolated with High Pure RNA Tissue Kit following the manufacturer's instructions. cDNA was transcribed with Roche Transcriptor First Strand cDNA Synthesis Kit following manufacturer's instructions.

PCR reaction

PCR reaction and reduction of data was performed based on the settings described in the product instructions (Table 2). The LightCycler® 480 Probes Master was used in the PCR reactions. Relative Cp values were calculated from expression levels of the reference genes.

Normalization of data

For normalization of the relative Cp values we used the procedure proposed by Vandesompele et al. by using GeNorm [4]. Correlation coefficient was determined by Spearman correlation in the statistics software.

Results and Discussion

Reproducibility

We performed all reactions in duplicate and examined relative Cp values of parallel measurements. We found high reproducibility and low standard deviance between parallel wells and values, except for a few obvious pipetting errors. One example is shown in Figure 1.

Table 1: Used cell lines, description and genetic status of main tumor genes.

Cell line	H358	A549	HCC827	H1993
ATCC ID	CRL-5807™	CCL-185™	CRL-2868™	CRL-5909™
Description	bronchoalveolar carcinoma	lung carcinoma	lung adenocarcinoma	lung adenocarcinoma
EGFR status	wild type	wild type	mutant (del19) amplification	wild type
CMET status	wild type	wild type	wild type	amplification
KRAS status	mutant (p.G12C)	mutant (p.G12S)	wild type	wild type
STK11 status	wild type	mutant (p.Q37*)	wild type	mutant (p.E199*)

Table 2: PCR program, recommended by Roche.

PCR-Protokoll (96 MWP)	°C	Slope*	Time	Acquisition
Denaturation	95		10 min	
Amplification	95	4,4	10 sec	
	60	2,2	30 sec	
	72	4,4	1 sec	Single
Cooling	40	2,2	30 sec	
Number of cycles				**45**

Differences in gene expressions

Our goal was to identify ABC transporters which are expressed in all lung cancer cell lines and also transporters with high variability. We have plotted the relative Cp values (normalized to the reference genes) and the percent of the variation coefficient (SD relative to the mean) (Figure 2). With this analysis we could identify 3 clusters of genes. The first group of genes are stably expressed in all cell lines (with less than 20% CV and less than 10 ΔCp), the second group consists the ABC transporters expressed at a very low level in all cell lines (less than 20% CV but higher that 15 ΔCp), the third group of genes show very high expression variability among the cell lines (more than

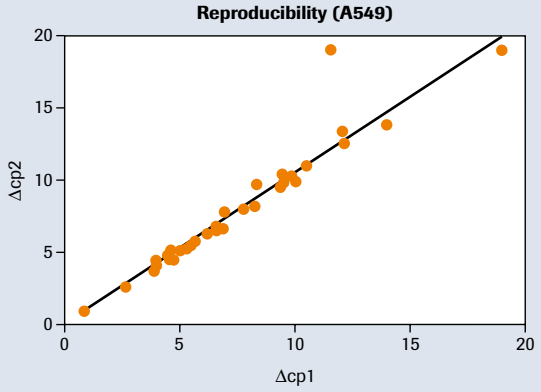

Figure 1: Reproducibility of measurements, correlating ΔCp1 with ΔCp2 of each measurement point in A549 cell line.

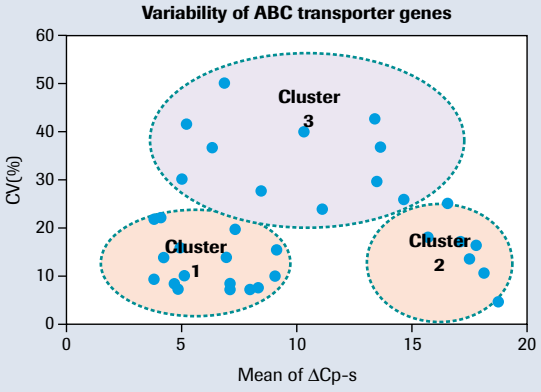

Figure 2: Mean of ΔCp values from all cell lines and all measurement points was correlated with the calculated coefficient of variation percentage.

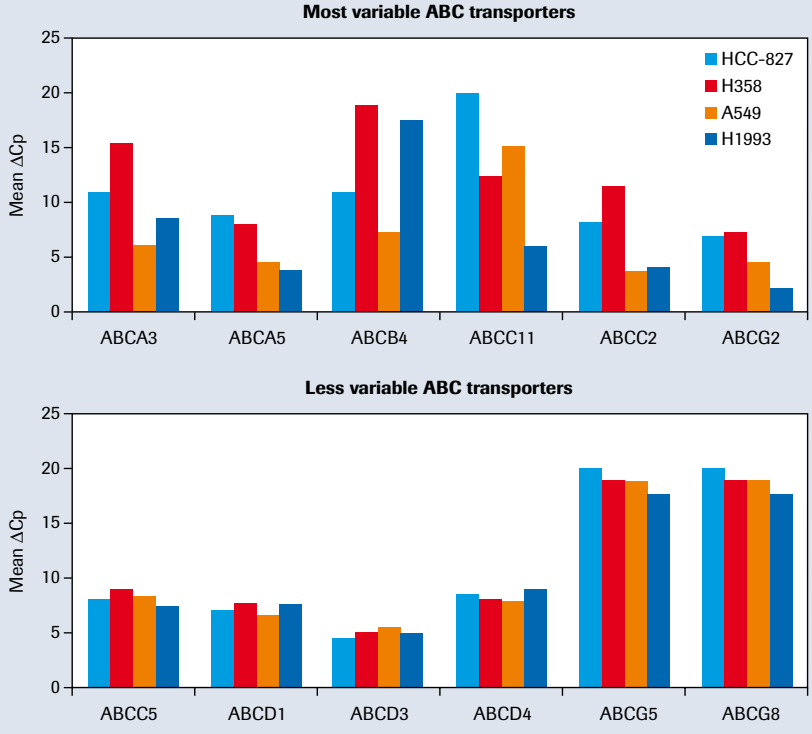

Figure 3: The 5 most and least variable ABC transporter genes. Mean ΔCp of ABC transporters are shown at all used lung cancer cell lines.

20% CV). We have also plotted the 5 most stable and the 5 most variable genes on bar diagrams (Figure 3).

Conclusions

Roche's RealTime ready ABC Transporter Panel is an easy-to-use, rapid system for expression profiling of ABC transporter genes. Because of its easy set up and operation, this system is recommended in studies of human ABC transporter genes. Simply adding the mixture of cDNA and PCR-master mix to the RealTime ready ABC Transporter Panel provided us with information about the expression level of all the relevant (42) ABC transporter genes in only 90 minutes on the LightCycler® 480 real-time PCR platform. We found the panel performance very robust, reliable and easy to use for our experiments.

These experiments provided novel data on the ABC transporter expression profile of NSCLC cells. Interestingly, these results are also in accordance with previous data, since the expression level of MDR1 (ABCB1) was also found to be very low or absent in research samples of NSCLC [5]. Our findings highlight the relevance of higher throughput expression analysis technologies in cancer research. ■

References

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Order INFO

Product	Pack Size	Cat. No.
RealTime ready ABC Transporter Panel, 96	2 LightCycler® 480 Multiwell Plates, 96	05 339 324 001
LightCycler® 480 Probes Master	5 x 1 ml (2x conc.)	04 707 494 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit 50 reactions	04 379 012 001
High Pure RNA Tissue Kit	1 kit, up to 50 isolations	12 033 674 001