

Microfluidic analysis of a multiplex RT-PCR assay for combined expression screening of melanoma drug resistance target genes

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Abstract

During the last decade, microarray analysis led to the identification of a large number of genes that are differentially expressed in disease processes. After validation, identified target genes can be either used for functional assays resulting in putative drug targets or for the screening of different samples (e.g. biopsies, cell lines, blood) in order to identify molecular markers for diagnostics. For a fast and reliable screening of multiple putative diagnostic markers involved in melanoma drug resistance, we developed an assay for a semi-quantitative expression analysis of 11 drug resistance target genes recently identified in a gene expression profiling study. A multiplex RT-PCR (mRT-PCR) assay was developed allowing the gene expression screening of 11 target genes and 1 housekeeping gene in one reaction. Combining mRT-PCR with microfluidic fragment analysis, we were able to resolve, size and quantify 12 mRT-PCR products ranging from 116–582 bp. To validate the quantitative performance of our assay, the determined expression ratios were compared with data from microarray- and Northern-blot analyses. 8 out of 11 target genes were reproducible in terms of expression ratios, indicating these genes as informative for screening approaches. Applied to 5 melanoma cell lines, the mRT-PCR assay clearly identified differential expression of 6 target genes.

In conjunction with recent data, the results point to the usability of APOD, CYR61 and IL1B as marker genes for etoposide resistance in melanoma cell lines.

Analytical Platform: Agilent 2100 bioanalyzer

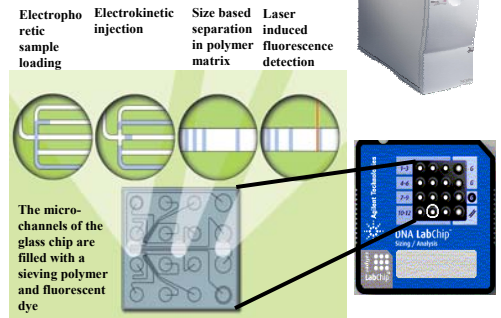
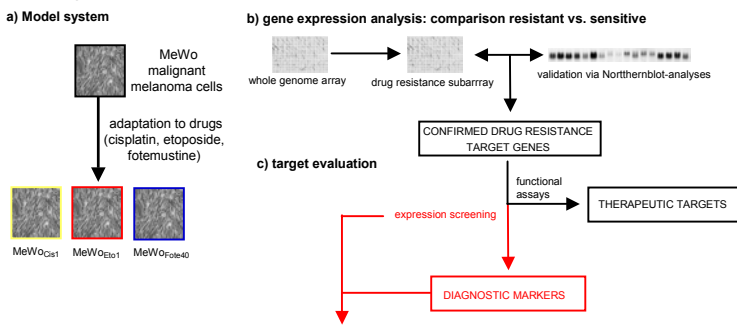


Fig. 1: Microfluidic analysis using lab-on-a-chip technology

Background



Development of mRT-PCR Assay for marker evaluation and diagnosis

Fig. 2: Schematic representation of the experimental set-up

Multiplex RT-PCR on the Agilent 2100 Bioanalyzer

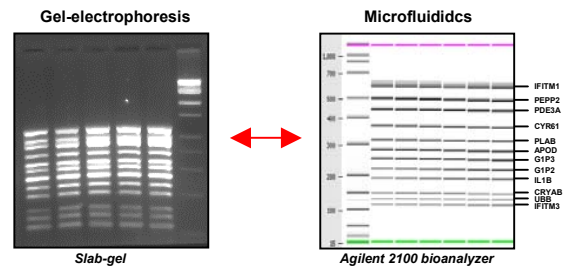


Fig.3 Development and analysis of a 12-plex mRT-PCR assay (Maxim Biotech).

A mRT-PCR assay was developed for the simultaneous expression profiling of 12 genes. The sizing resolution/accuracy of the slab gels as well as the Agilent 2100 bioanalyzer was compared by analyzing the 12plex RT-PCR. 7 out of the 12 PCR products are smaller than 300 bp. The electropherogram shows distinct peaks that are clearly distinguishable, whereas the fragment bands on the gel are merging and drifting.

Validation of mRT-PCR performance using a characterized in vitro model system for drug resistance

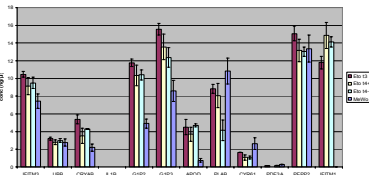


Fig.4A: Target gene expression levels (etoposide)

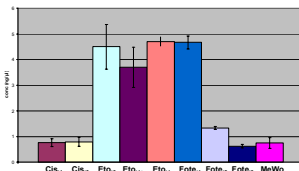


Fig.4C: APOD expression level of all melanoma cell lines

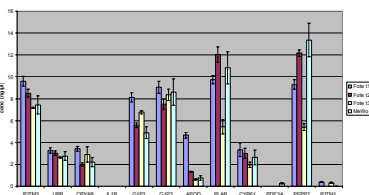


Fig.4B: Target gene expression levels (fotemustine)

The mRT-PCR assay was tested on cDNA of different drug resistant melanoma cell lines. Initial tests showed a high reproducibility in terms of fragment resolution, sizing and quantification (data not shown). The comparison of mRT-PCR expression patterns with microarray and Northern-blot results revealed data correlation for 9 out of 12 genes. Figures 4A+B show target gene expression levels of etoposide and fotemustine resistant melanoma cell lines. Figure 4C represents the expression level of APOD in all analyzed melanoma cell lines.

Summary

- Development of a 12plex mRT-PCR assay based on endpoint analysis
- Accurate mRT-PCR fragment resolution and sizing by using the Agilent 2100 bioanalyzer
- Highly reproducible quantification of endpoint mRT-PCR products
- Simultaneous expression profiling of 12 genes
- Expression patterns of 9 out of 12 genes validated with microarray and/or Northern-blot data
- MRT-PCR for screening of uncharacterized melanoma cell lines

Comparative expression analysis of additional Melanoma cell lines

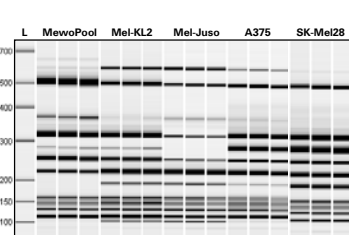


Fig. 5: mRT-PCR screening of 5 melanoma cell lines.

The mRT-PCR products assay were resolved and quantified on the Agilent 2100 bioanalyzer. The screening results revealed distinct differences in the expression pattern of 6 out of the 12 genes: IFITM3, PEPP2, CYR61, PLAB, APOD and IL1B.

Results (compared to MeWo)

- Upregulation of IL1B; highest expression in SK-Mel28
- Downregulation of PEPP2
- Downregulation of CYR61
- Downregulation of PLAB in Mel-Juso
- Upregulation of APOD in A375 and SK-Mel28
- Converse expression patterns of G1P2 and G1P3
- Uniform expression of CRYAB and UBB
- APOD, CYR61 and IL1B: etoposide resistance marker genes?

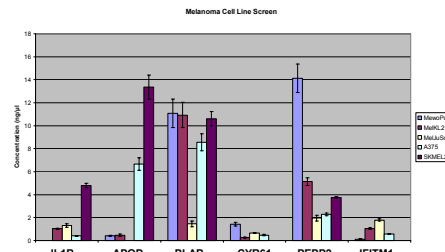


Fig. 6: Comparison of relative target gene expression levels.

Quantification of the mRT-PCR Products allowed the comparison of the relative expression patterns of the drug resistance target genes in 5 melanoma cell lines (e.g. upregulation of IL1B and APOD in SK-Mel28, downregulation of PEPP2 in 4 out of 5 cell lines). These highly reproducible results demonstrate the high potential of a combination of multiplex RT-PCR assay with an analytical platform using lab-on-a-chip technology