Reviewer's report

Title: Response rate of fibrosarcoma cells to cytotoxic drugs on the expression level correlates to the therapeutic response rate of fibrosarcomas and is mediated by regulation of apoptotic pathways

Version: 1 Date: 25 January 2005

Reviewer: Christopher CP Poremba

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In this manuscript, the authors perform Affymetrix HG-U133A based microarray gene expression profiling of the fibrosarcoma cell line HT1080 after treatment with actinomycin D, doxorubicin, or vincristin to get clues for the mechanism of induced cell death by these drugs. By statistical/numerical comparison with the untreated cells sets of genes could be identified which significantly are affected by either of these treatment regimes. For doxorubicine the authors could define a set of genes which clearly shows the involvement of different apoptotic pathways in tumour cell death.

The manuscript is well written and the aim of the project is of relevance to the medical research community. The data obtained by the GeneChip analysis may contribute to the understanding of mechanisms of chemotherapy in fibrosarcoma and serve as a starting point to further functional investigations. However, the study should be enhanced by the following items:

1. A direct correlation is suggested between the increasing number of differentially regulated genes for vincristin, doxorubicin, and actinomycin D and the clinical impact of these cytostatic drugs. This item should be handled by far more carefully:
   a. The effect of the different drugs is of course dependent on their concentration.
   b. Are any LD50-concentrations known for these drugs for HT1080?
   c. Which efforts have been made to compare cell vitality, drug concentration and changes in gene expression level?

2. In this study only one fibrosarcoma cell line was investigated. That means no information are available whether the behaviour of HT1080 is a typical one for fibrosarcoma or not. Therefore the data for HT1080 should not be generalised in an uncritical manner.

3. The authors state that 45 apoptosis genes were re-analysed by quantitative RT-PCR leading to a 62% success rate of validation. At this point more detailed information is needed: Which genes were analysed? How are genes leading to conflicting results handled in the GO-analysis? Are there hints which can explain the relatively high discrepancy between GeneChip data and real-time data?

4. In the discussion the authors deduce a cytochrom c release and a caspase activation from their gene expression data. How can the localisation or the enzymatic activity of a protein be determined by its mRNA abundance?

Additional minor points:

The differentially expressed genes in the actinomycin D experiments should be referred to as “under-represented” rather than “down-regulated” (see for example: Abstract/Results) since blocking the entire mRNA synthesis is not a regulatory event by the cell. In fact by this drug short-lived mRNA sub-populations will be erased more quickly in the cells than more stable mRNAs.

In the reference list many books are cited without editors and city

Legend to Fig 2 A-D: Do the data given in the scatterplots really represent 14,500 genes (as stated)
or more correctly, 14,500 probe sets? Were any row data excluded from this analysis?

**What next?:** Accept after minor essential revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests.