Author's response to reviews

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

Authors:

Marcus Lehnhardt (marcus.lehnhardt@rub.de)
Ludger Klein-Hitpass (Ludger.klein-hitpass@uni-essen.de)
Cornelius Kuhnen (Cornelius.Kuhnen@rub.de)
Heinz Herbert Homann (Heinz.Homann@rub.de)
Adrien Daigeler (Daigeler@hotmail.com)
Hans Ulrich Steinau (Hans-Ulrich.Steinau@bergmannsheil.de)
Laura Schnorr (Ulrich.Leyerer@t-online.de)
Sonja Roehrs (Sonja.roehrs@mpi-dortmund.mpg.de)
Lars Steinstraesser (Lars.Steinstraesser@rub.de)
Oliver Mueller (Oliver.mueller@mpi-dortmund.mpg.de)

Version: 2 Date: 22 March 2005

Author's response to reviews: see over
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

Dear Sirs,

We greatly appreciate the thoughtful review given our manuscript and have enclosed the revised manuscript # 4470114315741848.

Our point to point response to the critique follows below.

Thank you for considering this revised publication for BMC Cancer. Please let me know if I can provide any additional information.

Yours sincerely

Marcus Lehnhardt
Review: Abdelhadi Rebbaa

1. We included a further table (Tab. 3) to demonstrate quantitative data concerning the 46 selected genes validated by quantitative PCR. Further we addressed possible reasons for which can explain discrepancies between Gene Chip data and PCR data (see Results).

2. 1µg/ml was a false statement/transcriptipns error. The used doxorubicin concentration was 0,5µg/ml. This choosen doxorubicin concentration was derived from investigations concerning chemosensitivity testing in solid tumors. In this investigations 0,5 µg/ml doxorubicin is equivalent to the Test Drug Concentration of doxorubicin used in vitro. This concentration is supposed to reflect nearly the Plasma Peak Concentration in vivo (Untch M et al. Chemosensitivity testing in gynecologic oncology- dream or reality? Recent Results in Cancer Research 161, 146-158, 2003; Ugurel S et al. Chemosensitivity testing in malignant melanoma. Recent Results in Cancer Research 161, 81-92, 2003).

Furthermore high doxorubicin concentrations up to 1µg/ml are in common use in other cellular models (Kudoh K et al. Monitoring the expression profiles of doxorubicin-induced and doxorubicin-resistant cancer cells by cDNA microarrays. Cancer Research 60(15) 4161-6,2000).

We mainly wanted to compare expression profiles of HT1080 cells in response to the 3 drugs and therefore consciously choose this concentration for doxorubicin. Further investigation including different sarcoma cells, drug concentrations and time responses are needed. Because of limited supply it is not included in this study.

3. We appreciate this comment. The results demonstrated here relate to HT1080 fibrosarcoma cells only. Especially because of the heterogenous nature of soft tissue sarcomas our findings are not transferable to other sarcomas or solid tumors.

The manuscript was changed to emphasize this issue more clearly. In addition findings from this study are compared with other published array results in order to identify common genes that altered both fibrosarcoma and other types of cancer in response to this drug (see discussion).
Review: Christopher CP Poremba

1. b: The chosen drug concentrations were derived from investigations concerning chemosensitivity testing in solid tumors. In this investigations used concentrations are equivalent to the Test Drug Concentrations used in vitro. The concentrations are supposed to reflect nearly the Plasma Peak Concentration in vivo (Untch M et al. Chemosensitivity testing in gynecologic oncology- dream or reality? Recent Results in Cancer Research 161, 146-158, 2003; Ugurel S et al. Chemosensitivity testing in malignant melanoma. Recent Results in Cancer Research 161, 81-92, 2003).

c: We mainly wanted to compare expression profiles of HT1080 cells in response to the 3 drugs. We did not investigate cell vitality. Because of limited supply it is not included in this study.

2. We appreciate this comment. The results demonstrated here relate to HT1080 fibrosarcoma cells only. Especially because of the heterogenous nature of soft tissue sarcomas our findings are not transferable to other sarcomas or solid tumors. We changed the manuscript in order to reveal this issue.

3. We included a further table (Tab. 3) to demonstrate quantitative data concerning the 46 selected genes validated by quantitative PCR. Further we addressed possible reasons for which can explain discrepancies between Gene Chip data and PCR data (see Results). The 46 selected apoptosis genes re-analyzed by PCR are only in partial PP-calls in the microarray experiment. Thus we re-analyzed in part genes that were not securely detected by microarray. The overall success rate of validation for “hot targets” in the doxorubicin experiment was 78%.

   For GO-analysis only microarray-data were used.

4. We appreciate this comment and changed the manuscript therefore. The enzymatic activity of a protein cannot be determined by its mRNA abundance.

Additional minor points:

- expressed genes in the actinomycin D experiments referred to as “under-represented”
- the reference list was created using Endnote 6. We will ask the editorial board for different editing concerning editors and city
- the scatterplots represent 14,500 genes (22,800 probe sets). Row data were not excluded from this analysis.