Author’s response to reviews

Title: Natural resistance to ascorbic acid induced oxidative stress is mainly mediated by catalase activity in human cancer cells and catalase-silencing sensitizes to oxidative stress

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Version: 3 Date: 12 March 2012

Author’s response to reviews: see over
Cover letter with a point-by-point description of the changes made for the second reviewer reports

First, we would like to thank the reviewers for their helpful comments. The revised manuscript was corrected by a native speaker.

Reviewer Stanislav Janousek (Referee 1)
This reviewer has no questions.

Reviewer Yaxiong Tang (Referee 2)
1. More experimental data such as the effects of some other hydrogen peroxide-detoxifying enzymes are needed.
The manuscript focused data on the ability of human cancer cells to protect themselves against oxidative stress mediated by extracellular ascorbic acid-induced hydrogen production. Silenced catalase expression in the ascorbic acid-resistant cancer cell line BT-20 increased its susceptibility to oxidative stress (Fig. 6 and 7). In addition to catalase, enzymes of the peroxidase family, e.g. glutathione peroxidase, are also important for cell protection. The authors present data that the knock-down of catalase in BT-20 cells does not influence glutathione peroxidase activity (shown in additional Fig. 1). However, the uninfluenced activity of glutathione peroxidase was insufficient to protect catalase-negative BT-20 cells against ascorbic acid-mediated oxidative stress. This is in contrast to BT-20 wild type cells with uninfluenced catalase activity. In our mind these results are sufficient to hypothesize that glutathione peroxide play not a major role in protecting cancer cells against ascorbic acid-mediated oxidative stress.

2. We do not see a big difference in catalase activity from Fig. 4a and this indicated that catalase may not be the main target for ascorbic acid.
Fig 4a shows the catalase protein level normalized to β-actin and not the catalase enzymatic activity. The authors apologize for the misunderstanding Y-axis label. The authors changed the label from “Normalized catalase level” to Normalized catalase protein level”.

Since protein expression does not always correlate directly with enzymatic activity, the authors determine catalase activity (shown in Fig. 4b) which was significantly increased in the ascorbic acid-resistant cell lines SKOV-3, 23132/87 and BT-20 in comparison to the ascorbic acid non-resistant cell lines U-87 and U-251.

3. The manuscript should also provide the western blot results for some caspases.
The main message of the manuscript is that catalase is involved in the resistance of cancer cells to ascorbic acid mediated oxidative stress. Higher levels of catalase activity are found in cell lines that are resistant to oxidative stress than in more susceptible cancer cell lines. The investigation of caspase 3 and 7 activation represents only a minor part of the manuscript and the authors confirmed that ascorbic acid mediated cell death occurred by apoptosis. The activation of caspase 3 and 7 was analysed with the established luminescent assay provided by Promega. This assay is commonly used to analyse the activation of caspases (e.g. Gao P et al. J Biol Chem 2010; 285: 25570-25581)

4. In writing, detailed protocols need to be provided for all the data.
The protocols were completed. Established assays were used for analysing catalase activity and caspase activation and performed exactly according to manufacture’s instructions.
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Reviewer Hiroomi Tamura (Referee 3)
This reviewer has no questions.