Author's response to reviews

Title: Immunosuppressive Glycodelin A is an independent marker for poor prognosis in endometrial cancer

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Version: 2
Date: 21 October 2013

Author's response to reviews:

Dear Editors-in-Chief, Dear Editorial Board Members,

Thank you for your response regarding our manuscript entitled “Immunosuppressive Glycodelin A is an independent marker for poor prognosis in endometrial cancer”.

We are very grateful for the constructive comments we received regarding the above manuscript. We have carefully addressed the reviewers’ comments in the point-by-point response found below.

Thus we hope that our work is now considered suitable for being published as an original article in BMC Cancer.

Please do not hesitate to contact me in case of any questions regarding the manuscript.

Sincerely yours

Miriam Lenhard, MD

Point-by-point response regarding the reviewers’ comments

We again gratefully thank the reviewers for their constructive comments. Please find below our response to the comments raised.

Reviewer: Venkatakrishna Jala
Major Compulsory Revisions:

1. The manuscript describes GdA as an independent marker for poor prognosis in endometrial cancer. It is very interesting study and has importance in the field. However, there are major concerns, which authors need to address before its acceptance for publication.

2. Since the manuscript is completely based on IHC studies, authors should provide comprehensive IHC images to follow the MS description. Authors should provide representative images for the negative controls; IHC scoring; Undifferentiated cell staining patterns for Gd and GdA. Representative images for stage and their IHC staining for Gd and GdA. These can be included either in main text or in supplemental data.

Authors’ response: Representative microphotographs of positive vs. negative controls (Additional file 1), high vs. low IRS (Additional file 2), low vs. high FIGO stage (Additional file 3) and of a tumour of undifferentiated histology (Additional file 4) have been added as supplementary data, as instructed.

3. Please separate the images of IHC and in-situ hybridization and represent them as separate Figures. It is very difficult follow in the current form.

Authors’ response: Former figure 1 has been split into two separate figures, as instructed.

4. If possible, please provide some western blot data either cell lines or tissues of endometrial cancer to differentiate between expression levels of GdA and Gd. It would substantially increase the value of MS.

Authors’ response: In order to be able to include meaningful follow up data we analysed samples collected before 2002. Unfortunately, due to the very limited amount of tissue available, protein extraction on these samples cannot be performed.

However the differences between Gd and GdA have been intensively dissected in former studies published by our group (Jeschke et al. 2005; Jeschke et al. 2006). The analysis of GdA on western blots has also been already published (Jeschke et al. 2003, Jeschke et al. 2009) as stated in the manuscript.

5. Discussion needs to be modified and limitations of this study should to be discussed. Some of introduction and discussion have the same sentences (Page 3, 4th paragraph & page 11, 2nd paragraph). Please modify these parts.
Authors’ response: We thank the reviewer for this interesting comment. Comments on the limitations of the study have been added to the discussion, as instructed. The sentences referred to in the reviewer’s comment have been modified.

Minor Essential Revisions

1. Need to refer Figure 1 first in the text and then go to figure 2 (page 8, line 6 & 8)

Authors’ response: References to figures have been inserted in the correct order.

2. Please improve the quality of Figure 3. It is hard follow lines of Gd and GdA low.

Authors’ response: Quality of this Figure has been improved as instructed.

Reviewer: Hsien-Ming Wu

1. Glycodelin research has elucidated the biology of epithelial differentiation. The glandular association of native glycodelin indicates a fundamental role in glandular morphogenesis, and induced glycodelin expression in glycodelin-negative endometrial adenocarcinoma cells is associated with differentiation toward normal endometrial epithelium and restricted cell growth.

Drawing the protocol of using multiple endometrial cancer cells and the monitoring of the regulation of glycodelin will be more significant novel.

Authors’ response: Studies published by Ohta et al. 2008 and So et al. 2012 report growth and migration blocking abilities of Gd. Ohta and colleagues showed that overexpression of Gd reduced cell proliferation in the endometrial adenocarcinoma cell line Ishikawa. Our conclusions drawn from the survival analysis reported in the current manuscript are in line with these observations. We found that Gd positivity of the tumour is significantly associated with prolonged overall survival - a condition possibly related to tumour growth restrictive actions of Gd.

However it is much more challenging to investigate the role of GdA on cell growth. GdA may not directly affect downstream signalling pathways but may rather promote tumour growth by interfering with the body’s immune response.
To this direction it has been demonstrated that GdA inhibits the cytolytic activity of CD8+ T lymphocytes (Soni et al. 2010), has anti-growth activity and promotes apoptosis in monocytes (Alok et al. 2009). Animal models xenografted with tumours either depleted for GdA or overexpressing GdA would be needed to further clarify the role of GdA in modulating the host’s anti-tumour immune response.

2. In this manuscript, the authors mentioned that GdA holds prognostic significance for a poor outcome in endometrial cancer patients. This concept adds new knowledge to the gap of treatment for endometrial cancer. Further investigation into this exciting and multifunctional glycoprotein is bound to yield new insights into the treatment of endometrial cancer.

More studies employing downregulation of glycodelin by siRNA is to execute the differentiation- and cell attachment-enhancing actions. As a HDACI (SAHA) was recently approved by FDA for treatment of a malignant disease, research expanding this propensity into treatment of the hormone-related cancers remains a challenge for future studies. If the information about the concept can be added and discussed in this manuscript, this manuscript will do supply a major finding.

Authors’ response: We thank the reviewer for this very constructive comment. Recently histone deacetylase inhibitors (HDACIs) have been highlighted as promising new anti-cancer agents. In 2006 the HDACI suberoylanilide hydroxamic acid (SAHA, Vorinostat (rINN); Zolinza®) has been approved by the FDA for the treatment of cutaneous T-cell lymphoma and has further been evaluated in patients suffering from e.g. glioblastoma multiforme (Galanis et al. 2009), non-small-cell lung cancer (Traynor et al. 2009) or myelodysplastic syndroms (Garcia-Manero et al. 2012). Uchida et al. 2005, 2007a, 2007b demonstrated that SAHA is capable of up-regulating Gd in endometrial cancer and choriocarcinoma cell lines and further that SAHA induced Gd in fact influences cell differentiation and migration in the model system employed. Since we found that Gd is significantly associated with prolonged overall survival in endometrial cancer, it remains challenging to investigate whether endometrial cancer patients might also benefit from the application of SAHA. Of course randomized and properly powered clinical trials are indispensable in order to validate this hypothesis on a clinical basis.

A paragraph stating the above has been added to the manuscript text as instructed.

3. The current version of the manuscript raises some issues that need to be addressed to strengthen the manuscript and enhance its general interest and significance to be acceptable.

Authors’ response: We have carefully revised several passages in the manuscript in the aim of improving its general interest as instructed.