Author's response to reviews

Title: The antiapoptotic gene survivin is highly expressed in human chondrosarcoma and promotes drug resistance in chondrosarcoma cells in vitro

Authors:

Philipp Lechler (p.lechler@klepios.com)
Tobias Renkawitz (t.renkawitz@klepios.com)
Valentina Campean (Valentina.Campean@uk-erlangen.de)
Sanjeevi Balakrishnan (s.balakrishnan07@imperial.ac.uk)
Markus Tingart (mtingart@ukaachen.de)
Joachim Grifka (j.grifka@klepios.com)
Jens Schaumburger (j.schaumburger@klink.uni-r.de)

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Author's response to reviews: see over
Dear Sirs,

we thank the reviewers and the editor for their helpful comments; we feel that the resulting manuscript has improved substantially. In the following we have responded to the reviewer’s detailed comments by inserting our responses accordingly.

Referee 1:

Reviewer's report
Title: The antiapoptotic gene survivin is highly expressed in human chondrosarcoma and promotes drug resistance in chondrosarcoma cells in vitro

Version: 1 Date: 12 November 2010

Reviewer: Chih-Hsin Tang

Reviewer's report:
Accept

Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Referee 2:

Reviewer's report:
Lechler and colleagues present some very interesting results on the expression of survivin in chondrosarcoma of bone and their data suggest that its expression is related to chemoresistance. Since chondrosarcoma is highly resistant to conventional chemo- and radiotherapy, these are highly relevant findings. The experiments seem well performed and the manuscript is well written. There are however still some drawbacks of the manuscript:

1. the number of tumor specimens analyzed is rather small, while the number of high grade tumors is relatively large, therefore the results might not be representative for CS in general. The authors should explain why there is a disproportionate number of grade III chondrosarcomas, which are in general quite rare.

Response: We thank the reviewer for her most constructive comment on the revised manuscript. Still, high grade chondrosarcoma represent an unsolved oncologic challenge, while low grade chondrosarcomas can often be cured by complete surgical resection. Furthermore, the importance of histological grade is underlined by the fact that grade is directly related to metastatic rate and remains currently the single relevant predictor of patient outcome. Thus we focused the present study on grade II and III tumors. Obviously, future studies need to further dissect the relation between tumor grade, survivin expression and patient survival.
2. although results were obtained by different antibodies, the photographs of the immunohistochemistry reveal staining in all cells, including normal stromal cells and extracellular matrix surrounding the tumor. This is puzzling since surviving was claimed to have oncofetal expression, i.e. to be absent in normal tissue. The fact that expression was validated using western blot and RT-PCR is however reassuring.

Response: The reviewer emphasizes the importance of the application several different antibodies for immunohistochemistry. We confirmed the histological findings by the application of two independent polyclonal and 2 independent monoclonal antibodies. The reviewer states that all cells and even extracellular matrix stain positive for survivin. Figure 1 shows strong cellular expression of survivin protein in tumor cells and weaker expression in single surrounding cells. Chondroid extracellular matrix does not stain for survivin protein. High-power magnification reveals the predominantly cytoplasmic staining and strong survivin signals can be detected in tumor cells displaying mitotic figures. Latter is a well described sign of specificity of survivin immunohistochemistry (Li F, et al. Nature. 1998 10;396(6711):580-4., Uren AG, et al. Proc Natl Acad Sci U S A. 1999 31;96(18):10170-5. Li F, et al. Nat Cell Biol. 1999 1(8):461-6.) Survivin positive cells surrounding tumors and survivin positive tumor associated endothelial cells were previously described by other authors (e.g. Tran J, et al. Proc Natl Acad Sci U S A. 2002 99(7):4349-54., Virrey JJ, et al. Am J Pathol. 2008 ;173(2):575-85.). Survivin expression in the tumor environment might be driven by paracrine effect.

3. The same holds for the faint band seen in fig 1F; it seems that there is some expression in normal cartilage. In the discussion (although wrongly referred to fig 1D) this is acknowledged as such, although not explained, while in the results the 6 articular cartilages are stated negative.

Response: We thank the reviewer for the helpful annotations. We revised discussion and results.

4. the knock down of survivin is convincing at the mRNA level, however not at the protein level in SW1353.

Response: We agree with the reviewer that the knock down of survivin led to a more pronounced suppression of survivin on mRNA level than on protein level. Still, the reduction of survivin protein is obvious and the biologic effects on cell cycle and apoptosis were measurable by the application of independent test.

5. it is puzzling that despite the fact that there is knock down of survivin, there is still an increase in viable cells (fig 4A), although proliferation is reduced and there is apoptosis. The authors should comment on this.

Response: We agree with the reviewer that despite the transient knock down of survivin cell proliferation and viability are not completely blocked. Survivin is reported to be a crucial gene for mitosis and interference with survivin impairs mitotic cell division. Considering the subtotal knockdown of survivin protein as discussed in 4. and the marked prolifervative capacity of the analyzed tumor cell lines, neither a complete stop of tumor cell proliferation nor a total block in cell viability after the knock down of survivin are to be expected.

6. chondrosarcomas are considered a heterogeneous group of tumours which is also reflected by the different behaviour of the two cell lines (fig 6). The manuscript has benefited from the addition of an extra cell line, although the chosen cell line is not very well documented. It would be good to have more information on the Hs 819.T cell line since this is only poorly documented in literature and at the ATCC website. For instance, is extraskeletal myxoid chondrosarcoma excluded (i.e. absence of translocation)? This would be crucial.

Response: We thank the reviewer for the most constructive comment. We confirmed our in vitro
experiments in chondrosarcoma cell line SW-1353 by the use of a different cell line (Hs 819.T). Hs 819.T is commercially available (ATCC) and was previously described by Thein and Lotan (Thein R, Lotan R. Cancer Res. 1982 Nov;42(11):4771-5.) Here, the authors reported type of tumor, body part and patient details. Following studies analyzed the cell lines biologic behaviour (e.g. Human Molecular Genetic 13 (22): 2753-2765., The Journal of Clinical Endocrinology & Metabolism 88,(10): 4576-4585.) To our knowledge no informations concerning the absence of translocation yet exist.

Thus, results are interesting and relevant but should be interpreted with caution given the major drawbacks mentioned above.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests

Editor:
"I would like to give the authors the chance to respond to the reviewers' comments. I think that the immunohistochemistry is fine though no normal tissue is shown (which should be negative)."
Response: Immunohistochemistry was performed according to the protocol described in detail in the methods section. Four independent primary antibodies were applied, of which two were of monoclonal origin. Omission of primary or secondary antibody resulted in completely negative tissue sections. Normal physiologic cartilage sections were processed in parallel and showed no staining after the application of all four primary antibodies.