Author's response to reviews

Title: Epigenetic silencing of genes and microRNAs within the imprinted Dlk1-Dio3 region at human chromosome 14.32 in giant cell tumor of bone

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Comments to the reviewers

Reviewer 1:

Comment:
Do Aza and PBA increase the expression of these genes through targeting IG-DMR CpG 1-13 that the author found as a frequent hypermethylation in GCTSCs?

Response:
Both, the demethylating agent Aza and the histone deacetylase inhibitor PBA are unspecific inhibitors that do not act in a sequence specific way. Therefore, all analyzed sequences are equally influenced by Aza and PBA treatment. Our conclusion from this experiment was that DNA methylation is involved in the regulation of the analyzed genes, but we cannot conclude that gene expression is targeted only by the IG-DMR CpG 1-13 region. However, the fact that this region is frequently hypermethylated in GCTSCs as shown in figure 6 strongly suggests an involvement of this region.

Comment:
What is the influence of the up-regulation of these genes to GCTSCs, as there is still few reports about them in GCT?

Response:
We agree that the analysis of the functional impact of the observed upregulation of the analyzed genes is a very important step. So far, we observed a marked decrease in proliferation of GCTSCs treated with Aza and PBA. However, as explained above Aza and PBA treatment lead to the upregulation of many genes and possible functional differences cannot be attributed to the analyzed genes. Generation of stable transfected cell lines, overexpressing the candidate genes would be necessary for such investigations. This will be done in future projects. In our opinion, addition of such data would exceed the scope of the current
Reviewer 2:

Comment:
These are unclear concept and it is not according to the general concept of the benign and malignant tumor. Metastasis is a phenomenon associated to tumors with malignant phenotype, or in other words, only malignant tumor present potential to gives Metastasis. We suggest that phrase showed be rewritten accordingly, as follow:
"Despite their benign nature, GCTs are characterized by a highly variable and unpredictable behavior. Although rare, GCT can manifests a malignant phenotype, and metastases has been described in up to 5% of the cases [3,4]."

Response:
The sentence has been rewritten accordingly (page 3, line 56-57)

Comment:
Complete the references

Response:
The references have been completed (page 4 and 5, line 96, 98, 100 and 106)

Comment:
Complete the references according to former citation

Response:
The reference has been completed (page 5, line 107-108)

Comment:
Ethic concerns should be put in the begining or in the ending of the MM section.
“Ethics Committee of the University of Heidelberg”

Response:
The sentence has been moved to the beginning of the Materials and Methods section and was rewriting (page 4, line 89-90)

Comment:
Complete the citation

Response:
The citation has been completed (page 5, line 113)

Comment:
We encourage the autors to use the standardized nomenclature for q-PCR and related techniques suggested by Bustin et al. Clin Chemistry 55: 4, 2009.

Response:
The term „quantitative RT-PCR“ was replaced by „RT-qPCR“ throughout the text.

Comment:
These point have been not informes in the MM section.
Response:
The method has been described in a previous publication already cited in the text. The sequence of the primers used to verify the purity of the isolated GCTSCs were included in the Materials and Methods section (page 6, line 140-143).

Comment:
This information should be given at the begiing of the MM section in order to organize the information on the text.
Response:
Information on patient characteristics in table 1 was moved to the beginning of the “Materials and Methods” section (page 4, line 90-91).

Comment:
All the reasons that motivate authors to use the present methods should be expressed on the Introduction section or on the Discussion section to give more fluency to the Text.
Response:
In our opinion, the short link to our previous study given in the results section is important for the reader to better understand our study design, the importance of figure 1 and the results of the experiment described after this section. A more detailed explanation of our previous data and methodology was already given in the introduction.