Author's response to reviews

Title: BMP and TGFbeta Pathways in Human Central Chondrosarcoma: Enhanced Endoglin and Smad 1 Signaling in High Grade Tumors

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Reviewer: John Healey

1. Correction for multiple comparisons must be made, or a convincing justification for why it is not necessary. For example, data dredging like reporting for each chondrosarcoma grade and then lumping all grade 2 and 3 tumors together is questionable and generates different statistical results for different parameters. This calls the overall analysis into question.

We revised the statistical analysis of our data according to the reviewers comments. In order to simplify the presentation of the results in the new version of the manuscript we concentrated on important comparisons and introduced Bonferroni correction keeping clinical relevance in mind. Gene expression analysis (figure 1) now considers only comparisons between grade 1, 2 and 3 individually plus the comparison of all chondrosarcoma together (all grades) with normal cartilage. In line with this reviewers comments (see revision 1) potential differences in gene expression between healthy cartilage and single chondrosarcoma groups was considered to be of minor interest and removed.

For the immunohistochemical data, a statistical comparison between all single grade chondrosarcoma groups was removed due to the number of available samples of the clinically very rare grade III chondrosarcoma (n=6), which is too low for a chi-square test. We have now limited the analysis to the comparison between low-grade (grade I) and high-grade (grade II + III) chondrosarcoma since this is also clinically justified according to the different clinical behavior of grade II and III chondrosarcomas versus grade I. This is a common approach that was followed previously in well-respected studies (Schrage et al., Am J Pathol. 2009;174:979-88; Hameetman et al., J. Pathol. 2006;209:501-11).

This is now also explained in the methods’ section.

2. The p 10 revision “Therefore, the hypothesis can be made that endoglin could represent an important mediator of tumorangiogenesis in high-grade chondrosarcoma. The analysis of the correlation between endoglin expression and tumor vascularization would allow to establish whether a link between these pathways could exist in central chondrosarcoma.” This is true.
So much so that I think the additional work should be done to make this a paper that tests an hypothesis rather than just generating hypotheses.

The second sentence stated above is unfortunately a bit misleading as it implies a simple way to establish an association between endoglin expression and tumor vascularization. It is known that high grade chondrosarcomas demonstrate increased microvessel density (Ayala et al., Hum. Pathol. 2000;31;341-346; Boeuf et al., Histopathology. 2010;56:641-51) and this phenomenon is also clinically used in dynamic MRI and to diagnose chondrosarcoma. If we would stain our chondrosarcoma samples for CD31 and assess microvessel density, we would find increased microvessel density with increased histological grade as reported in the literature. We would probably find an association between microvessel density and endoglin expression, but there would be no way to prove that these two phenomena are causally related. No immunohistochemical analysis would allow to establish such a causal link.

An association between angiogenesis and endoglin expression could therefore only be approached in vitro in chondrosarcoma cells and animal models. To prove a link between the pathways would however require a sophisticated setup including silencing and xenografts. This would represent a new project in itself and cannot be treated as a supplement to this manuscript.

The misleading sentence in the discussion (p.10) has been modified.