Reviewer's report

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

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General comments:

The expression of BMP and TGFβ genes was measured in 26 central chondrosarcoma and

6 normal cartilage samples by quantitative RT-PCR. Expression of endoglin and nuclear localization of phosphorylated Smad1/5/8 and Smad2 was assessed by immunohistochemical analysis. The expression of TGFB3 and of the activin receptor-like kinase ALK2 was found to be significantly higher in grade III compared to grade I chondrosarcoma. Nuclear phosphorylated Smad1/5/8 and Smad2 were found in all tumors analyzed and the activity of both signaling pathways was confirmed by functional reporter assays in 2 chondrosarcoma cell lines. The authors conclude that immunohistochemical analysis furthermore revealed that phosphorylated Smad1/5/8 and endoglin expression were significantly higher in high-grade compared to low-grade chondrosarcoma and correlated to each other. This is an interesting topic about which little has been done. Understanding the progression of chondrosarcoma is important in a pathophysiological sense in because the clonal progression seen in many tumors suggests that there may be a certain stepwise progression in this cancer that is not seen in other sarcomas. The other fashion in which progression is considered, increased invasiveness, metastasis, and lethality are not addressed in this manuscript. The implications for the findings are poorly analyzed, in a somewhat random laundry list fashion, and the main conclusion that angiogenesis is in somehow related to the Smad1/5/8 and endoglin expression profiles is without any experimental support.

The action and pathways for BMP’s and TGFbeta are beautifully described. Although somewhat long, the description is essential to help the reader understand the point of this paper.

The only ligand that was expressed differently between high and low grade tumors was TGFB3, but it was at only 2.2-fold difference of expression.

The results comparing cancer to normal cartilage were expected and not very useful, other than as a negative control for the assays.

The finding of increased endoglin with higher grade tumors was not quite statistically significant, and was also somewhat an expected result. This
correlation was not independent from the histopathological grade of the tumors. Thus is not going to be a helpful diagnostic tool nor a prognostic tool. Its value is unclear.

The cell line data are rather tangential and certainly can’t reflect on the endoglin axis directly.

Finally, the statistical analysis is not defined. Were there appropriate corrections made for multiple comparisons? Ultimately, a more systems biology approach should be taken for these axies.

Specific Review questions:

1. Is the question posed by the authors well defined? Well enough
2. Are the methods appropriate and well described? Yes.
3. Are the data sound? Yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes
5. Are the discussion and conclusions well balanced and adequately supported by the data? Mostly, but speculation about vascularization is not supported.
6. Are limitations of the work clearly stated? No.
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes.
8. Do the title and abstract accurately convey what has been found? Yes
9. Is the writing acceptable? Yes

Specific questions about the manuscript:

1. Introduction “a pivotal regulator providing a link between the undifferentiated phenotype of tumor cells in high-grade chondrosarcoma and the angiogenic status of these tumors.” This was not proven by this investigation, so it should not be emphasized here. Indeed, on page 6 you state “Only expression in tumor cells and not in the vasculature was scored in this study”

2. P 4. “In central chondrosarcoma, active TGF# signaling has been shown according to nuclear phosphorylated Smad2 detection, and the expression of PAI1, a target gene of TGF#Smad2/3, was shown to be higher in high grade tumors [10].” This makes no sense. Please rewrite more clearly.


4. P12. Was the RNA prepared at each center and then combined for analysis, or was the tissue processed centrally in the same lab?