Reviewer's report

Title: Characterization of the Interleukin-17 Effect on Articular Cartilage in a Translational Model. An Explorative Study

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Reviewer: Frank Zaucke

Reviewer's report:

In this manuscript, the authors aimed to characterize the role of IL-17 in articular cartilage degeneration. This cytokine has been shown to be upregulated in both serum and synovial fluid of osteoarthritis (OA) patients. There is a correlation between IL-17 levels and degeneration but the effect of IL-17 on cartilage extracellular matrix turnover, in particular in healthy cartilage, has not yet been studied in sufficient detail. Due to the limited availability of healthy human samples the authors used bovine articular cartilage explants that were treated with IL-17 or a combination of oncostatin M and TNF, respectively. Standard biochemical assays were used to quantify GAG and ECM turnover. Specific proteins and fragments thereof were detected and analysed by shotgun LC MS/MS.

In principle, the study is interesting, technically well-performed and clinically relevant. However, there are a couple of points that could, to my mind, improve the manuscript significantly and that the authors might want to consider:

1. In 'background' the authors first describe OA that might also have an inflammatory component and that there is some overlap with rheumatoid arthritis (RA). However, it remains a bit unclear if IL-17 levels were shown to be increased in OA. If so, what are the (patho)physiological concentrations and when in the process of degeneration were increased levels detected. In most of their experiments two concentrations are used but the basis for selecting these concentrations is not given. It could also make sense to check if the effects of these two concentrations are significantly different in any of the experiments performed (e.g. Figure 4C and D?). When looking at Figure 3C, there seems to be more PG retained in the sample treated with 25ng/ml IL-17. However, this is not reflected by the quantification in A or B.
2. The legend of Figure 1 has to be improved. In how many samples (cows) did the authors try to detect the different isoforms? A negative result does not necessarily mean that the isoform is not expressed as long as the authors do not have good controls. Further, I would be very careful with IL-17RE. Without sequencing, I am not convinced that the arrows points to the right product.

3. Why did the authors select MMP2 and -9? And did not include MMP-1,-3 and -13 which are known to be induced by IL-17 (line 404)? Further, in earlier studies, others have shown that IL-17 effects are mediated by aggrecanases and NOT MMPs. The authors should discuss their own results in the context of these findings. They could also make more clear what their major advance in knowledge is compared to earlier studies using porcine explants.

4. The authors discuss limitations of their study appropriately. I can confirm from my own experience that it is difficult to find good and specific antibodies against bovine proteins. However, I think that a validation of the data presented in Figure 6A and B with immunoblots or -stainings would substantially improve the quality of the manuscript.

Minor points:

The term 'hind knees' is unusual. Would 'knee' not be sufficient to identify the joint that was analysed?

I assume that the oxygen concentration used was 20%. As a lot of cartilage research is performed under hypoxia or physioxia I would recommend mentioning the oxygen concentration.

What is GIMP2?

I am uncertain if I can see a small gradual reduction in metabolic activity (line 319). Over the culture period. Metabolic activity and cell viability are of course two different things. Is it known if the treatments affect cell proliferation, viability and thus metabolic activity per cell?

Does O+T treatment affect IL-17 signalling?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes
**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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