Reviewer’s report

Title: Human MMP28 expression is unresponsive to inflammatory stimuli and does not correlate to the grade of intervertebral disc degeneration

Version: 1 Date: 9 May 2011

Reviewer number: 3

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Major Compulsory Revisions

1. Specimens from the same patient, same disc level and same degeneration grade should essentially be considered as one sample. It is evident that in most cases these samples also show very similar gene expression levels. In view of this fact the sample number is only 2, 1, 3, and 2 for each of the degeneration grades. This makes it extremely difficult to draw a relevant conclusion, taking into account the inter-donor variations even within the same group. This should be addressed in the discussion.

2. The sample size could be significantly increased if the basal MMP28 expression of the disc cells isolated for the second part of the study could be included in the first part of the study. This would additionally provide information about the expression of MMP28 in herniated discs.

3. Knowing that the Thompson grade generally shows a positive correlation with the age of the patient, it seems surprising to see young patients with grade IV-V, while the oldest patient showed the lowest degeneration level. While this may be observed occasionally, it is certainly not common and should be taken into consideration. Potential correlation of MMP28 expression with the age of the patient could also be considered.

4. To demonstrate that MMP28 in fact seems to be regulated differently from other MMPs the expression of (an)other MMP(s) known to be increased in degenerative discs should be determined in addition to MMP28.

5. Similarly, to demonstrate that the IVD cells were responsive to the treatment with the inflammatory mediators, the expression of (an)other MMP(s) known to be increased by these agents should be shown. As in herniated discs the level of inflammation may already be quite high the cells may be less responsive.

Minor Essential Revisions

1. In the methods part, more detailed information about the cell stimulation conditions with respect to cell density (or level of confluence) is required.
2. In most cases total RNA is isolated and not mRNA as described in the methods part. It is also assumed that 1µg of total RNA (and not mRNA) was reverse transcribed.

3. According to the methods section the 2(-ddCt) method was used for calculation of gene expression levels. However these values are not displayed in the graphs. Negative values can only occur when displayed in a logarithmic scale. If log-scale is shown this needs to be specified in the legend and/or graph.

4. For the first study apparently the 2(-dCt) method was used for calculation of gene expression levels. This needs to be added to the methods part.

5. In figure 1 the authors should specify whether MMP28 was not detected at all in some patients or whether the expression was lower than 1. If the latter is the case, logarithmic illustration of the data would be recommended. Then it would also be found that the variations among the grade II samples are in fact relatively small, i.e. only about a 3 times difference between the lowest and highest value.

6. The authors refer to the findings of Gruber et al. in the discussion, where MMP28 precursor expression was found up-regulated in non-degenerated discs. In the methods section it should thus be specified whether the expression of MMP28 or of MMP28 precursor was measured. Moreover, while the data referenced originate from annulus cells, the authors did not further define their samples, although differences between annulus and nucleus cells are described. At least where available the origin (NP or AF) should be noted.

Discretionary Revisions

1. Background: Some additional information about the MMP28 substrates and function, if available, would be of interest with respect to the IVD homeostasis.

2. "altered expression of MMP28 during trauma and certain cases of more severe degeneration" is described in the discussion section. The authors need to consider that normal healthy disc cells were not analysed and therefore "altered" expression cannot be clearly defined.

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

**Quality of written English:** Acceptable

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