MSCs Secretome for Intervertebral Disc: from therapeutic targets to a functional role in an ex vivo pro-inflammatory model of IVD degeneration

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Abstract

Intervertebral disc (IVD) degeneration is considered the major cause of spinal disorders [1] and is associated with loss of ECM components (namely type II collagen and aggrecan) [2]. As a pro-inflammatory environment caused by the increased production of factors such as IL-1β [3]. Although mesenchymal stem/stromal cells (MSCs) regenerative potential has been demonstrated in this tissue, it may be hampered by the harsh, hypoxic, avascular and pro-inflammatory environment of the degenerated IVD in vivo [4]. This work focused on the effect of the secretome of pre-conditioned MSCs (with hypoxia and IL-1β) in a pro-inflammatory/degnerative IVD organ culture from bovine origin [5].

Conclusions

- The percentage of CD44+ cells is higher in the degenerative IVD, indicating that these could be explored as therapeutic targets for IVD degenerative disease.
- MSC-Cec has an immunomodulatory effect on the degenerative IVD.
- MSC-Cec increases aggrecan degradation in the degenerative IVD. An increase in BMP2 along with changes in the BMP/SMAD dynamics due to the presence of the secretome may be in the genesis of this effect.

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References


Figure 1. Characterization of IVD cell populations

The degenerative IVD cells were analyzed for the expression of CD44, CD45 and CD68 after dithiothreitol (DTT) treatment. After sorting, flow cytometry and FACS analysis were performed, and expression of major cytokines (IL-1β, IL-6, TNF-α) was analyzed using qRT-PCR. An immunohistochemical staining was performed using a monoclonal antibody directed against CD44, CD68 and CD45. A Representative of immunohistochemical staining shows the cell distribution of CD44, CD68 and CD45 (scalebar, 14 μm). B: Quantification of cells stained positive for CD44, CD68 and CD45. Results were presented as mean ± SD. C: Representative of flow cytometry plots showing the cell distribution of CD44, CD68 and CD45 (scalebar, 14 μm). D: Quantification of cells stained positive for each of the markers, individually or simultaneously.

Figure 2. Effect of pre-conditioning on MSCs composition

The composition of BM-MSCs (proliferative and non-proliferative) was analyzed in the pre-conditioned and non-pre-conditioned samples. A: Graphical representation of the experimental setup. B: Representative images of flow cytometry plots showing the cell distribution of CD44, CD68 and CD45 (scalebar, 14 μm). C: Quantification of cells stained positive for each of the markers, individually or simultaneously.

Figure 3. MSC-Cec effect on degenerative IVD inflammation and matrix degradation

A: Graphical illustration of experimental setup. B: After 24h, RNA expression of pro-inflammatory cytokines (IL-6, IL-8, matrix metalloproteinases (MMP-9, MMP-13) and their inhibitors (TIMP-1, TIMP-2)) gene expression levels were assessed and normalized to β-actin. The results are presented as fold change to control IVDs control levels (1 ± 0). All the results were presented as box and whisker plots with representation of median, interquartile range and maximum values and statistical analysis was performed using non-parametric paired Wilcoxon test. (n=5, *P<0.05, **P<0.01). C-D: Expression of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, MMP-9, MMP-13, TIMP-1, TIMP-2) was measured by ELISA and Western Blot. E: Graphical representation of the experimental setup. F: Graphical representation of the experimental setup.