Differences between healthy and degenerated intervertebral disc cells metabolic response to Bone Morphogenetic Proteins' Influence

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Introduction: The IVD intervertebral disc (IVD) is the largest avascular body organ. Decreased nutrient diffusion is a known cause of IVD degeneration. Recently suggested strategies for IVD regeneration include treatment with growth factors, of which the family of Bone Morphogenetic Proteins (BMP) have significant regenerative potential. We have previously studied the effect of BMPs on a healthy IVD metabolism, however their effect on degenerated IVD cells is unknown. In this study we determine the effects of three types of BMPs on the healthy and degenerated IVD cells' protein production and glucose consumption.

Materials and Methods: Human annulus fibrosus (AF) and nucleus pulposus (NP) cells were isolated from the degenerated disc tissue obtained during discectomy (Degenerated group) or from a 24-week fetus (Healthy group, from ScienCell). The cells were cultured in 1) control medium, and 2) medium supplemented with BMP-2, 3) BMP-7, and 4) BMP-14 100 ng/ml. Trypan blue, alamarBlue®, glucose, lactate, and 1,9-dimethyl-methylene blue (DMMB) assays were performed. Cell morphology was studied using immunocytochemistry: cells were fixed and stained with phalloidin for F-actin and DAPI for nuclear DNA, and imaged using a laser confocal microscope. Cell culture experiments were performed in n=3 replicates for degenerated and n=6 replicates for healthy subgroups totaling 72 samples.

Results and Discussion: Degenerated IVD cells were significantly less proliferative than healthy (p<0.01 in all NP groups and p<0.01 in all AF groups), however the differences in viability did not reach significance. BMP 2, 7 and 14 had a tendency to increase the cell viability in a degenerated NP cells comparing to a control NP group. (p=0.12, p=0.04, p=0.04 for BMP 2, 7 and 14 respectively).

Cultured IVD cells stained for actin microfilaments and nuclei and imaged with a confocal microscopy were spindle shaped with long protrusions. Cell morphology did not appear qualitatively different between corresponding NP and AF cell types; however, healthy cells had more robust and apparent cytoskeletons.

Glucose consumption and lactate production did not differ significantly between healthy and degenerated cells in all groups except those supplemented with BMP 2 where cells had decreased glucose consumption (NP cells, p=0.03) and lactate production (AF cells, p=0.05). Overall glycosaminoglycan production was significantly lower in degenerated NP and AF cells when compared to the healthy groups, p<0.01 for both cell types. In degenerated cell groups BMP 2, 7 and 14 were able to increase glycosaminoglycan production compared to the control (p=0.13; p=0.05 and p=0.05 for BMP 2, 7 and 14 respectively) but did not reach the rate of the healthy cells (figure 1).

Metabolic activity did not differ significantly in degenerated and healthy AF cells, but was significantly higher in degenerated NP than healthy NP cells (p=0.001) Metabolic activity was not increased in the BMP groups, when compared to control.

Conclusions: Bone morphogenetic proteins' potential as regenerative therapies are supported by their influence on viability of the degenerated NP cells, extracellular matrix synthesis by degenerated NP cells rather than their stimulation of cell proliferation. The changes in IVD cells’ nutrient consumption under the influence of growth factors favors their application in combination with cell transplantation and requires further investigation.

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