

The impact of signaling factors on intervertebral disc degeneration and regeneration- Studies on disc and mesenchymal stem cells from chronic low back pain patients

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentligen försvaras i **R-aulan**, Mölndal sjukhus, Göteborgsvägen 31, Mölndal, **fredagen den 5 juni 2020**, kl. **09:00** av

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Avhandlingen baseras på följande delarbeten

- I. Hingert D, Barreto Henriksson H, Brisby H. Human Mesenchymal Stem Cells Pretreated with Interleukin-1 β and Stimulated with Bone Morphogenetic Growth Factor-3 Enhance Chondrogenesis. *Tissue Eng Part A*. 2018 May; 24(9-10):775-785. doi: 10.1089/ten.TEA.2017.0087
- II. Hingert D, Nilsson J, Barreto Henriksson H, Baranto A, Brisby H.. Pathological Effects of Cortisol on Intervertebral Disc Cells and Mesenchymal Stem Cells from Lower Back Pain Patients. *Cells Tissues Organs*. 2019;207(1):34-45. doi: 10.1159/000500772.
- III. Hingert D, Barreto Henriksson H, Baranto A, Brisby H. BMP-3 Promotes Matrix Production in Co-cultured Stem Cells and Disc Cells from Low Back Pain Patients. *Tissue Eng Part A*. 2020 Jan; 26(1-2):4756.doi:10.1089/ten.TEA.2019.0125
- IV. Hingert D, Nawilajaroen P, Aldridge J, Baranto A, Brisby H: Investigation of the Effect of Secreted Factors from Mesenchymal Stem Cells on Disc Cells from Degenerated Discs. *Cells Tissues Organs* 2020. doi: 10.1159/000506350
- V. Hingert D, Nawilajaroen P, Ekström K, Baranto A, Brisby H. Human levels of MMP-1 in degenerate discs can be mitigated by signaling peptides from mesenchymal stem cells. Submitted.
- VI. Hingert D, Ekström K, Aldridge J, Crescitelli R, Brisby H. Extracellular vesicles from human mesenchymal stem cells expedite chondrogenesis in 3D human degenerative disc cell cultures. Submitted.

**SAHLGRENKA AKADEMIN
INSTITUTIONEN FÖR KLINISKA VETENSKAPER**



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Abstract

INTRODUCTION: Chronic low back pain (LBP) is associated with degeneration of the intervertebral discs (IVDs). Increased expressions of pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and matrix metalloproteinases (MMPs) in degenerated IVDs lead to loss of proteoglycan and extracellular matrix (ECM) which affect the viability of the disc cells (DCs). Treatment approaches using growth factors, cell therapy and extracellular vesicles (EVs) derived from human mesenchymal stem cells (hMSCs) could improve current treatment models by directly influencing the IVD degeneration processes.

AIMS: To explore the effects of growth factors, hMSC derived signaling peptides and small EVs (sEVs) on degenerated DCs in terms of cell viability and ECM production and to investigate the impact of stress hormone cortisol on DCs and hMSCs in *in vitro* models.

METHODS: DC and hMSC isolation from patients' tissue, cell cultures in monolayer and 3D pellets, cell viability assay, histological staining, and immunohistochemistry were carried out. In Study I, hMSCs were encapsulated in a hydrogel and stimulated with bone morphogenetic growth factor 3 (BMP-3), or IL-1 β pre-treatment followed by BMP-3 stimulation. *In situ* hybridization was used to investigate the gene expressions of COL2A1 and OCT4. In Study II, the effects of cortisol at physiological and increased levels were studied on DCs and hMSCs in the 3D pellet model. Apoptosis assays were carried out and immunohistochemistry was used to evaluate cytokine expressions. Study III was a follow-up study of Study I in a 3D pellet model investigating the effect of BMP-3 and pre-treatment on DCs, hMSCs and co-culture (DCs and hMSCs in 1:1 ratio). In Study IV, the effects of hMSC conditioned media (CM) and connective tissue growth factor (CTGF) were investigated on DC pellets. The constituents of CM were further identified using mass spectrometry analysis. In Study V, the concentration of MMP-1 was quantified by enzyme-linked immunosorbent assay in disc tissue. Furthermore, the ability of CM to mitigate the effects of MMP-1 at different concentrations was studied. In Study VI, small EVs were isolated with differential centrifugation, and further characterized using flow cytometry, nanoparticle tracking analysis, and western blot. DC pellets were then stimulated with sEVs and cell proliferation, ECM production, apoptosis, lactate dehydrogenase activity, cytokine and chemokine secretions were evaluated.

RESULTS: Pre-treatment of IL-1 β followed by BMP-3 enhanced chondrogenic differentiation in hMSCs in the hydrogel model (Study I) as well as in the 3D model (Study III). BMP-3 promoted chondrogenesis in DC pellets while a stronger effect was observed in co-culture (Study III). Study II demonstrated that exposure to cortisol even at physiological concentration restricted proliferation and compromised chondrogenesis in both DCs and hMSCs. CM from hMSCs enhanced viability and ECM production in DCs and mass spectrometry analysis revealed more than 120 peptides with high relative abundance (Study IV). Study V demonstrated that CM has the ability to mitigate the effect of MMP-1 on DCs, however, the potency of CM decreased with increased concentration of MMP-1. Lastly, Study VI demonstrated that sEVs enhanced cell proliferation while suppressed apoptosis. Early and increased ECM production was also observed in the DCs with sEVs treatment.

CONCLUSION: Signaling factors from hMSCs have positive effects on DCs and can mitigate the degenerative properties of pro-inflammatory cytokines and enzymes known to be present in the degenerated IVDs. Further, pain- induced stress regulated by cortisol may be a contributing factor of IVD degeneration.

KEYWORDS: signaling peptides, MSCs, disc cells, BMP-3, IL-1beta, cortisol, co-culture, conditioned media, MMP-1, extracellular vesicles, chondrogenesis, low back pain.

ISBN: 978-91-7833-852-8 (TRYCK)

<http://hdl.handle.net/2077/63609>

ISBN: 978-91-7833-853-5 (PDF)