A Comparison of Bovine Adipose-Derived Mesenchymal Stromal Cells With Nucleus Pulposus Progenitor Cells: Exploring Extracellular Vesicle Therapy for Intervertebral Disk Regeneration

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Introduction

- Low back pain (and associated intervertebral disc (IVD) degeneration) is the most common health problem worldwide and leads to an immense social and financial burden. [1,2]
- Recently discovered progenitor cells in the IVD, the so-called Nucleus pulposus (NP) progenitor cells (NPPC), might be used for cell-based therapies. [3]
- These NPPCs are positive for the receptor TIE2 (aka. Angiopoietin-1 receptor/ CD202b).
- Recent study suggests NPPC derived extracellular vesicles (EVs) promote degenerated NP cell proliferation in vitro and attenuate IVD degeneration in vivo. [4]

Study Aims

To characterize and compare the EVs secreted from three different primary cell sources and their cells using transcriptomics and proteomics.

1. **Bovine TIE2+ NP cells**
2. **Bovine TIE2- NP cells**
3. **Bovine adipose derived mesenchymal stromal cells (AdMSCs)**

Materials and Methods

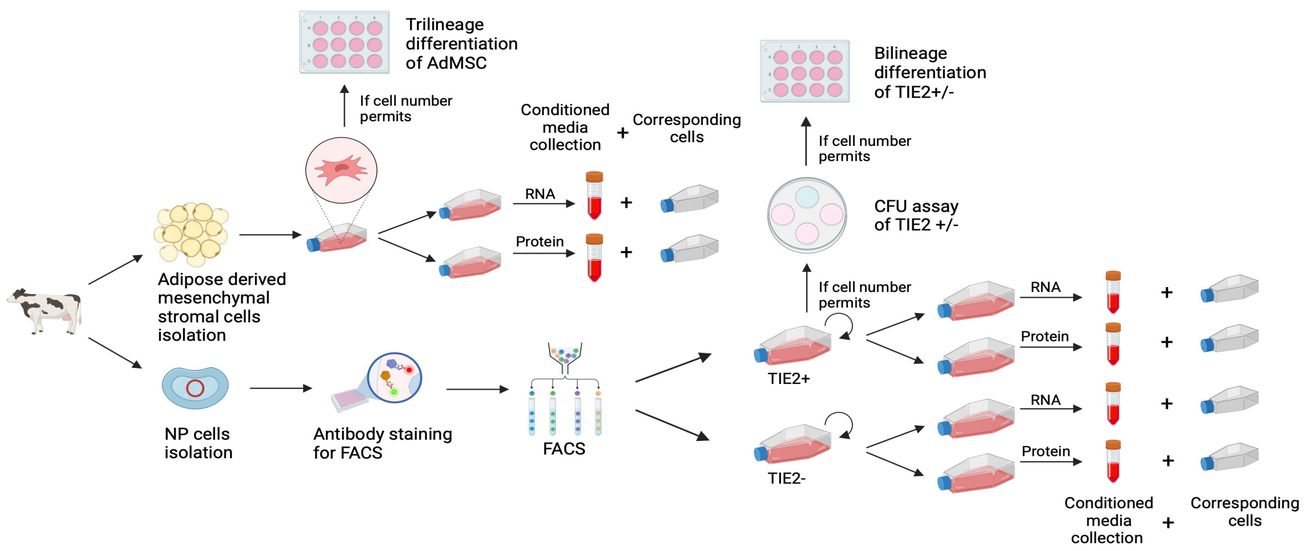


Figure 1: Graphical study design: Overview of the isolation of adipose-derived mesenchymal stromal cells (AdMSCs) and nucleus pulposus cells (NPCs) from bovine tissue, followed by fluorescence-activated cell sorting (FACS) for NPCs to obtain TIE2+ and TIE2- cell populations. After expansion, cells and conditioned media (extracellular vesicles) were harvested for proteomics and transcriptomics analysis. Additionally, functional assays were performed: a colony forming unit (CFU) assay and a bi-lineage differentiation assay for NPCs, as well as a trilineage differentiation assay for AdMSCs. Figure created with BioRender.com

Results

CFU Assay

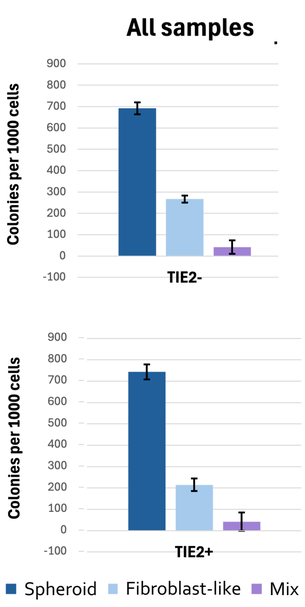


Figure 2, CFU assay: Pooled samples show ratio of spheroid, fibroblast-like and mixed phenotype colonies formed per 1000 cells by TIE2+ and TIE2- NP cells. Mean ± SD, n=4, CFU = Colony forming unit

Chondrogenic Differentiation

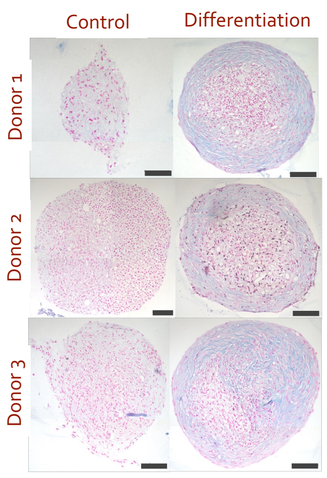


Figure 3, Differentiation assay: Pellets obtained from chondrogenic differentiation assay of three AdMSCs donors. Pellets were stained with Alcian blue and nuclear fast red, scale bar= 100 µm.

Adipogenic and osteogenic differentiation did not work as expected (data not shown).

Proteomics

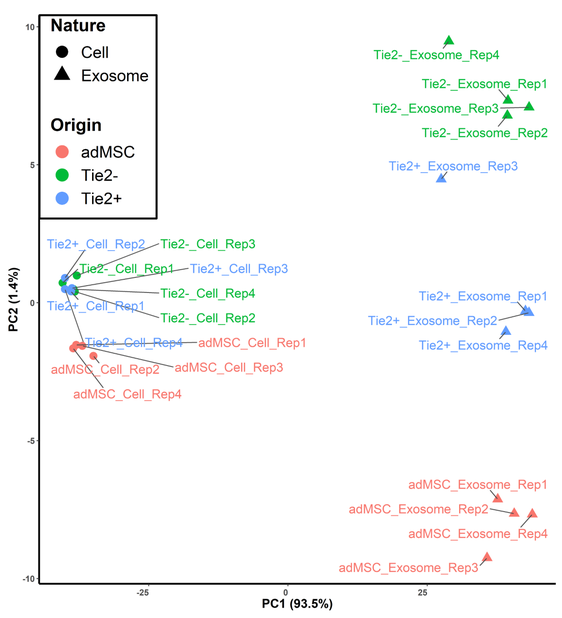


Figure 4, Principal component analysis (PCA) of the log2-transformed iSN intensities using the top 20% most variable proteins: Proteomics of TIE2+ and TIE2- NP cells and AdMSCs and their EVs.

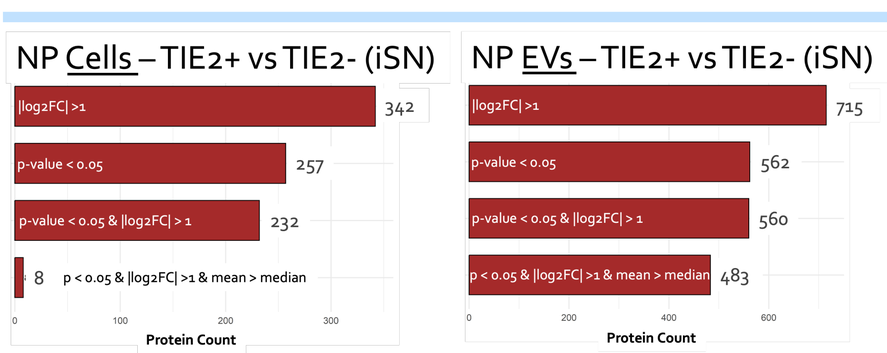


Figure 5, Protein count of differentially expressed proteins in TIE2+ vs TIE2- NP cells and EVs: Different filter criteria lead to different number of differentially expressed proteins. The number of highly differentially expressed proteins is different from cells to EVs.

Transcriptomics

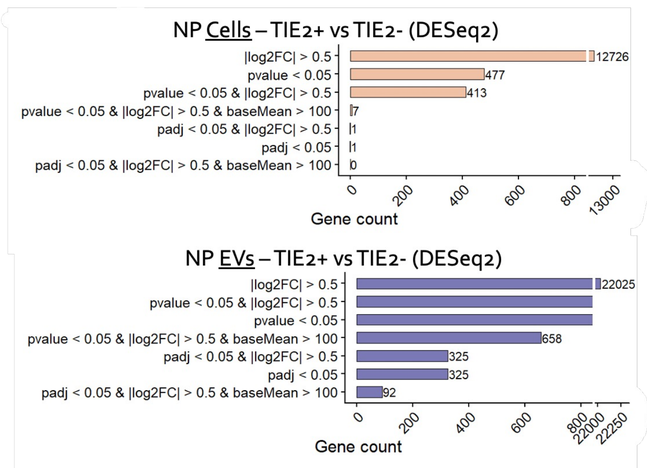


Figure 6, Gene count of differentially expressed genes in TIE2+ vs TIE2- NP cells and EVs: Different filter criteria lead to different number of differentially expressed proteins. The number of highly differentially expressed genes is different from cells to EVs.

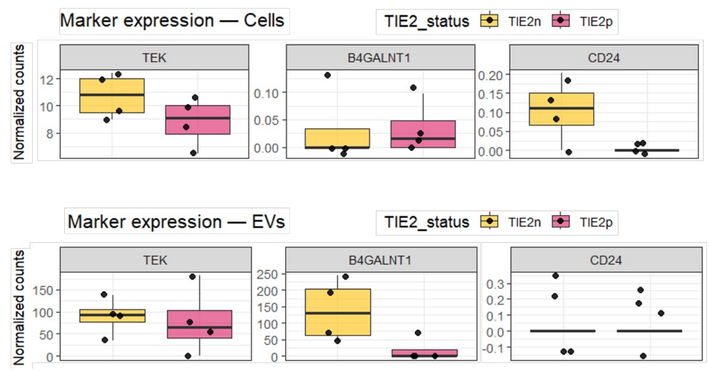


Figure 7, Gene expression: *TEK* showed no difference between cell types or EVs, though it appeared more abundant in EVs. CD24 mRNA was only detected in TIE2- NP cells , and B4GALNT1, encoding the GD2-synthesizing enzyme, was modestly upregulated in EVs from TIE2- NP cells compared with TIE2+ NP cells.

Discussion

- Successful isolation of the different EV types form TIE2+ and TIE2- NP cells and AdMSCs.
- CFU assay revealed no difference between TIE2+ and TIE2- NP cells.
- Bilineage differentiation of NP cells did not work as expected. Trilineage differentiation of AdMSCs assay showed chondrogenic differentiation.
- EV characterization showed slight variations between EVs subtypes (TIE2+ and TIE2- NP Cells, AdMSCs).
- Proteomics showed clear differences between protein and gene expression of cells and Evs.
- While TIE2+ and TIE2- NP cells seem to be similar, their EVs are different, both on protein and gene level. However, the progenitor status of the TIE2+ NP cells is unclear

Outlook

- Increase EV yield
- Western blot (e.g. CD9, CD63, CD81)
- Electron microscopy
- Live cell imaging to investigate uptake of EVs by cells.
- Compare results with recent study on bovine NP cell derived EVs. [5]
- Investigate potential use in IVD regeneration: Investigate their regenerative properties in a bovine ex vivo IVD degeneration model.

References

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Online Poster

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