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Bulk RNA Next Generation Sequencing of Native Donor-matched Human Tie2+ Progenitors versus Tie2- Cells

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INTRODUCTION: Stem cell therapy of the degenerate and painful intervertebral disc (IVD) is one of the most warranted but also highly disputed procedures to be applied in patients with low back pain. A particular autochthonous progenitor cell population has been identified in the center of the IVD, so-called nucleus pulposus (NP) progenitor cells (NPPC), positive for angiopoietin-1 receptor (= CD202b, Tie2, TEK gene). Here, we present novel Next Generation Sequencing (NGS) data and compare the transcriptome of FACS-sorted Tie2+ NPPC with Tie2- IVD cells and unsorted native cells. Furthemore, we explored whether expansion in presence of bFGF2 influenced the transciptome.
METHODS: Primary cells were isolated from N = 32 donors with written ethical consent of the Insel University hospital (Swissethics # 2019-00097). Inclusion criteria were 18-70 years of age, males and females alike undergoing spinal surgery. Cells were isolated from trauma tissue by 1h pronase followed by collagenase 2 digestion overnight. The cells were then expanded on plastics in normoxia and low-glucose DMEM + 10% FCS ± 2.5μg/mL bFGF-2. When passage 0 was 90% confluent, the cells were trypsinized, labelled using Tie2+ antibody, and then sorted using a FACSARIAIII. For bulk RNA-sequencing we isolated 17 high quality total RNAs at passage 2 Tie2± cells and from passage 1 unsorted donor-matched cells from 5 donors using a column purification method. All RNA was checked for integrity RIN > 7.0 and absence of contaminants. RNA sequencing was performed at the Functional Genomics Center Zürich. Extracted RNA was prepared for sequencing using the Illumina TruSeq Stranded mRNA Library Prep assay.
RESULTS: There was no significant correlation between donor age and the percentage of NPPC Tie2+cells in 1st passaged cell populations (N = 32 patients). In two donors, no Tie2+cells could be observed. Transcriptomics showed distinct clustering of samples that were either Tie2+ sorted, Tie2- or unsorted. Tie2+ selected cells revealed 551 differentially expressed genes (DEGs) ($p < 0.001$) compared to Tie2-cells; KRT19, a previously described marker for “nulceopulpocytes”, was significantly upregulated in Tie2+ cells; ACAN, PEG3, IGF2BP3, SERPIN1 and PRG4 (lubricin) were significantly down-regulated. GO Pathway analysis showed an enrichment of matrix genes and genes involved in cell cycle control and matrix production.
DISCUSSION & CONCLUSIONS: Here, new “molecular markers” for Tie2+ cells were identified.

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