Native Tie2+ Progenitor Cells - The ultimate Rejuvenation Source for the Degenerated Intervertebral Disc?

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INTRODUCTION

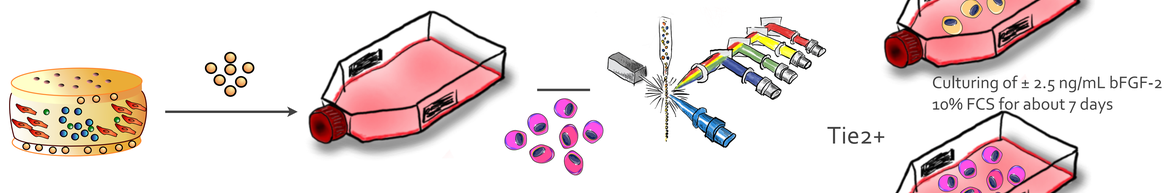
- Angiopoeitin receptor 1 (aka Tie2+) nucleus pulposus progenitor cells (NPPC) have been described by Daisuke Sakai and his team and by our group from the non-degenerative intervertebral disc (IVD) from human, mice, and cattle [1,2].
- Here, we present evidence for Tie2+ cells from primary clinical data of human IVD tissue, and passage 1 FACS-sorted cell's data for Tie2+ (aka CD202b) marker.
- How Tie2+ cells differ with respect to gene expression in their phenotype to other IVD cells is currently unknown. Here, we present first next generation sequencing (NGS) data comparing IVD cells, i.e. Tie2± NPC and Tie2± AFC, of both clonal, and non-clonal origin.

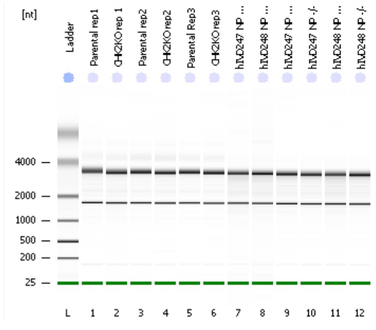
Study Aim

- to compare the transcriptome of unmodified Tie2+ NPPC with Tie2- IVD cells and unsorted cells.
- **Sub-aim**: Compare the effect of adding the growth factor during expansion bFGF2 onto the transciptome.

MATERIALS and METHODS

- Isolation of Primary Cells from Trauma Patients aged 18-60 yrs with written consent





Quality and integrity check of purified total RNA, RIN >7 are sent to NGS facility.

Output Parameters

- Relative gene Expression of passage 2 Tie2+ vs Tie2- and mixed IVD cell populations using bulk-RNAseq.
- Data analysis and visualisation using statistical analysis of differently expressed genes (DFGs) and KEOG pathway analysis.
- 20Mbp reads were conducted.

RESULTS

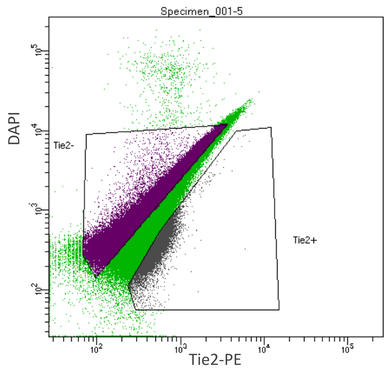


Figure 1: Typical FACS Sorting gating after primary cell sorting. The FLowJo™ plot depicts NPC of a single donor , i.e. donor 245 here with, 20,000 cells of ~0.5 M total isolated cells after enzymatic digestion and po expansion.

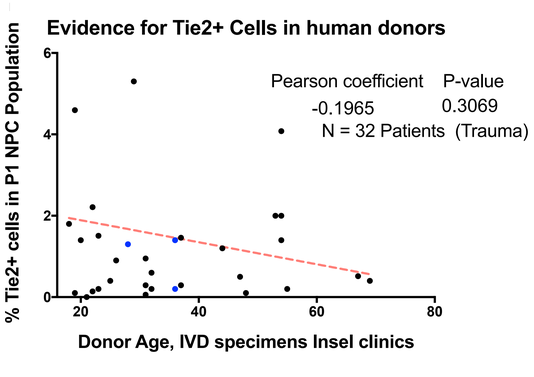
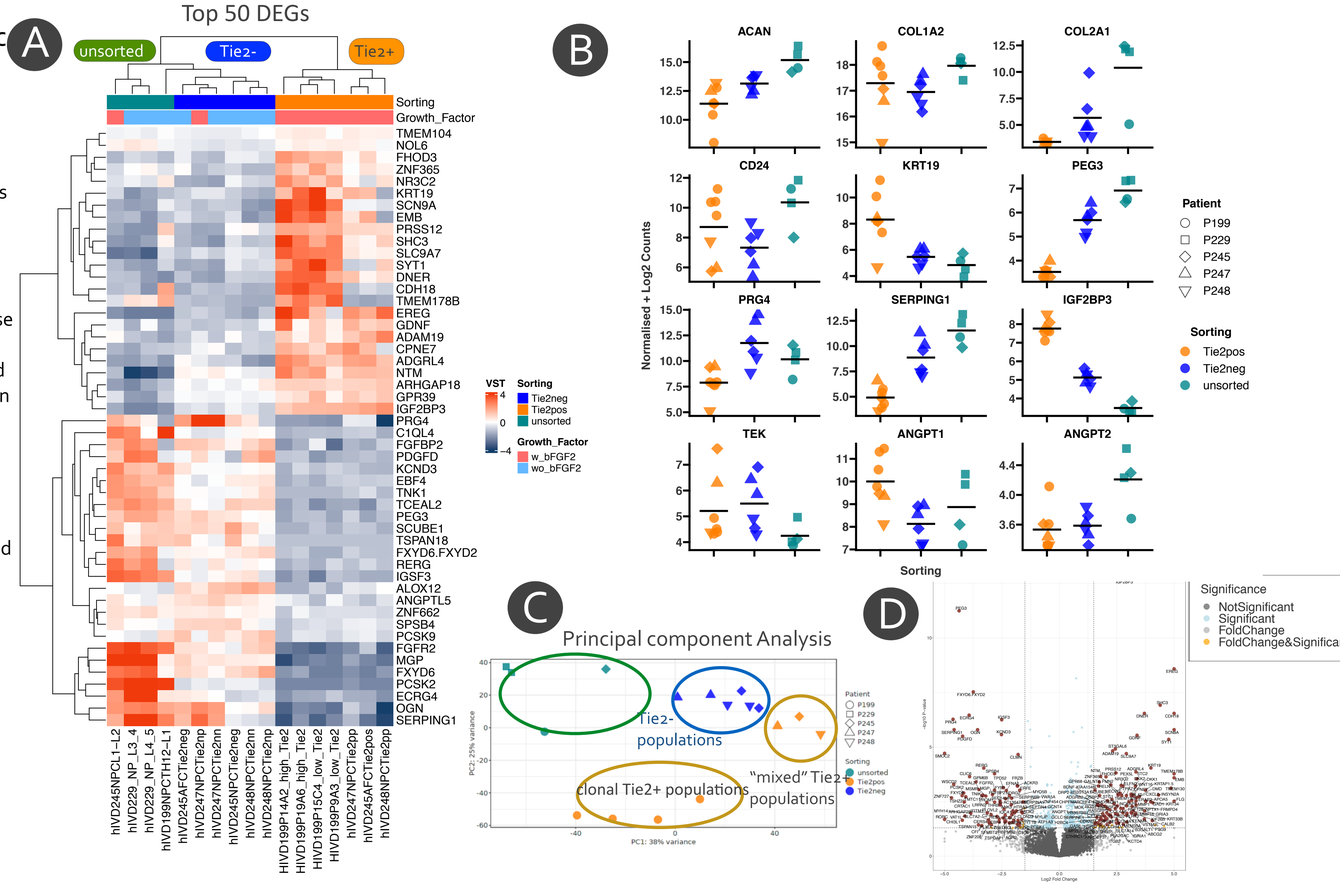


Figure 2: Current evidence of the percentage of Tie2+ NPPC detected with cell sorting by flow cytometry in primary NPC examined in passage 1 on standard lab plastics versus donor age.

Figure 3: Results of NGS sequencing of primary Tie2 NPPC A) Dendrogram of top 50 up-or down-differentially expressed genes (DEGs). Tie2+ cells were isolated by FACS sorting from passage 0 cells, then expanded to passage 1, sorted and re-seeded as clones with critical dilution. Tie2- and unsorted mixed population from the same donor. The data confirmed that cell sorting is a critical factor for clustering of these NPPC cells and that these are different from mixed unsorted and Tie2- cell populations. The addition of bFGF-2 did not seem to have a critical effect of changing the transcriptome **B) Selected box plots C) Principal Component Analysis**
D) **Volcano plot** between Tie2+ and Tie2- cell populations.



- Tie2+ cells could be successfully isolated from 32 donors (18-69 yrs).
- There was no correlation between donor age and percentage of Tie2+ cells (Figure 2).
- The NGS data of currently 17 samples from five trauma donors showed a clear clustering of samples that were either Tie2+ sorted, Tie2- or the unsorted population (Fig. 3). bFGF2 did not have a significant effect in the cultures.
- PCA demonstrated that clonal Tie2+ cells from one donor differed from other Tie2+ cells that were not cultured as single clones.

DISCUSSION

- The number of Tie2+ per disc per donor is highly variable and cell yield is somewhat unpredictable from donor age and/or sex (Figure 2)
- Low-Passage of Tie2+ selected cells on standard plastics and subsequent bulk RNAseq of poly-AAA mRNA revealed many DEGs; among them KRT19, a previously described marker for nulceopulpocytes [3] ,was found significantly unregulated in Tie2+ cells: PRG4 (lubricin) was found significantly down-regulated (Fig. 3A, B).

REFERENCES

[1] Sakai et al. (2018) *JOR Spine 1(2):e1018.*
[2] Tekari et al. (2016) *Stem Cell Res Ther 7(1):75.*
[3] Gantenbein-Ritter et al. (2011) *Eur Spine J 20: 962-971.*

ACKNOWLEDGEMENT

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