Exploring a Novel Spheroid 3D Cell Culture System for Tie2$^+$ Nucleus Pulposus Cells of the Intervertebral Disc

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Introduction

Low back pain (LBP) is a big problem in today's Western European society[1]. LBP impacts the patient's quality of life and places an immense burden on the healthcare system worldwide[2,3]. Degeneration of the intervertebral disc (IVD) is one of the common causes of LBP[4]. Recently, nucleus pulposus (NP) progenitor cells (NPPC) were discovered, which are positive for Angiopoietin-1 receptor (aka. Tie2/CD202b). These NPPCs are a promising cell source for IVD regeneration[5,6]. NPPCs are rare (2-10% of all IVD cells) in human IVDs and diminish in number with increasing age[6]. It has been demonstrated that 3D culture is superior to classic 2D culture to maintain the pluripotent phenotype of the NPPCs[7-9]. The goal of this research project is to test the expansion and culture of bovine NPPC in a novel spheroid plate.

Methods

NP tissue was isolated from bovine tails (aged 10-14 months). NP cells were isolated by using a mild two-step digestion protocol. Then, the primary NP cells were sorted by fluorescence activated cell sorting (FACS). NP cells were expanded for two weeks under hypoxia (2% O_2) and were supplemented with 2.5 ng/ml basic fibroblast growth factor 2(bFGF2) (fig. 1). Tie2$^+$ and Tie2$^-$ cells were then seeded in the functionalized and the 2D control wells of the Sphericalplate 5D (SP5D) of Kugelmeier, Ltd (fig. 2). The cells were cultured for two weeks under hypoxia and with bFGF2. A colony forming unit-assay was performed on day 0. Cell activity, DNA/GAG content and gene expression of selected genes were measured on day one, eight, and 15.

Results

Cell isolation from NP tissue yielded on average 15.7 million primary cells, of which ~10 k cells (0.063%) were positive for Tie2. Assessing the colony-forming ability of expanded cells revealed no significant differences between Tie2$^+$ and Tie2$^-$ cells. Cells in 2D plastic control wells proliferated significantly more compared to the spheroids ($p = 0.0002$). Analyzing gene expression after expansion revealed a five-fold increase of KRT19 and a four-fold increase of SOX2 in Tie2$^+$ cells in relation to Tie2$^-$ cells. Gene expression of KRT19 and SOX2 was found to be significantly downregulated in Tie2$^+$ cells in spheroid and 2D culture after 15 days (fig. 3).

Discussion

The results indicate that sorted NPPSs differentiated during expansion, resulting in a low share of Tie2$^+$ cells. Unexpectedly, successive culture in the SP5D revealed that such expanded Tie2$^+$ cells could not be kept in a non-differentiated stem-cell like state although such speriod-like cultures have been promising for many stem cells from various tissues and also in Tie2$^+$ cells[7,8]. The biological nature of the NPPCs are undeniably a promising approach for therapy of the degenerated IVDs.

Acknowledgments

This research was supported by project a Swiss National Science Bridge Discovery Project # 40B2-0_211510/1 (https://data.snf.ch/grants/grant/211510).

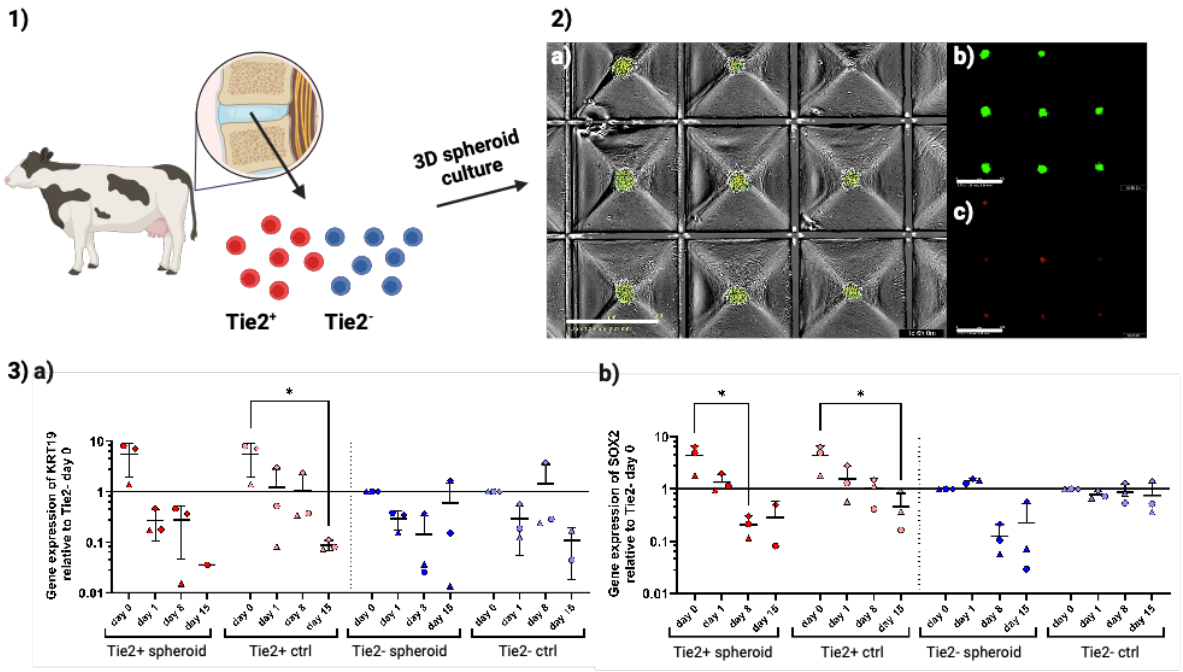

Fig. 1: Methods: Isolation of primary nucleus pulposus cells from bovine tails (aged 11-14 months), FACS for Tie2 and after 2D expansion culture in 3D spheroid plates. **Fig. 2**: Tie2- spheroids (400 cells) in microwells of SP5D, 1d 6h after seeding. Imaged with IncuCyte S3. (a) combined pictures of phase contrast, red and green fluorescent channel (b) green channel: Calcein AM = living cells (c) red channel: ethidium homodimer = dead cells. 10x magnification; scalebar = 400 µm. **Fig. 3**: qPCR results of (a) KRT19 and (b) SOX2, mean ± SD; N = 3; p-value < 0.05 = * ; Created with BioRender.com

Keywords

Intervertebral Disc Research, Nucleus Pulposus Progenitor Cells, Tie2, Angiopoietin-1 receptor, Spheroid Culture.

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