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Editorial

New Developments on Growth Factors, Exosomes, and Single Cell RNA-Sequencing for Regeneration of the Intervertebral Disc

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1. Introduction

Low back pain (LBP) is the number one cause of disability worldwide, with incidences increasing exponentially [1–3]. A recent study estimates that by the year 2050, an increase of 200 million people is expected, with a current peak at 619 million people [1]. This Special Issue targets the specific niche of finding innovative ways to address the clinical problem of LBP, which is often induced by the prolapse of the spinal column caused by genetic or epigenetic factors. Intervertebral disc (IVD) degeneration is often believed to be the root cause of chronic pain. Future research aims to understand the contribution of metabolic factors such as nutrition, besides other risk factors such as smoking and *Diabetes mellitus* [1]. This Special Issue provides a heterogeneous snapshot of recent applied research on IVD and LBP, ranging from cell biology studies to artificial intelligence in diagnostics. In the following subsections, I provide a short overview and summarize the seven articles' main findings.

2. Wet Laboratory Studies in the Second Special Issue

Two original studies focused on the single-cell transcriptomics to characterize phenotypes in rats in an IVD degeneration model [4] or performed a pathway study of ~100 genes using qPCR gene arrays using total RNA extractions from human donors suffering from idiopathic skeletal hyperostosis (DISH). The first study established an in vivo IVD degeneration model in 8-week-old Sprague Dawley rats that underwent surgery for retroperitoneal exposure using a 27 Gauge needle of the L4-L6 lumbar spine. Rohanifar et al. (2022) [4] then allowed the rats to recover for two and eight weeks postoperatively. Then, they digested the single cells from the tissue using mild digestion protocols, extracted and sequenced the total RNA and compared the next-generation sequencing (NGS) data relative to untreated controls. Rohanifar et al. (2022) confirmed that the nucleus pulposus (NP) mainly expressed key markers such as CD24 [5] as well as aggrecan and collagen type 2 [6–8]. In the outer part of the IVD, the cells mainly expressed collagen type 1, as previously identified in rats [6–8] but also in other species such as bovine and human [9]. Furthermore, their data allowed us to distinguish IVD cells from lymphoid, endothelial and myeloid cells [4]. The needle-punctured groups subsequently had a significantly higher amount of myeloid cells and lymphocytes than controls. These data are logical since needle puncture causes inflammation [4]. The second transcriptome study is on DISH patients. DISH is also known as Forestier's disease [10–13]. To the best of my knowledge, the study by Gantenbein et al. (2021) [14] is the first to elucidate on the possible phenotypic changes and deregulations of DISH cells compared to the phenotype of IVD cells isolated from trauma patients. This comparison has its limitations, of course, as trauma cells are not necessarily "healthy" cells. However, it most likely that these cells are still in a better state than cells

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isolated from degenerated IVDs. Nevertheless, by comparing the expression levels of bone morphogenic protein pathway cytokines and their inhibitors, i.e., Gremlin, Noggin and Chordin, my group found that these were dys-regulated [15], although these changes were not significant. However, DISH-IVD, in contrast to IVD obtained from trauma, showed a significant up-regulation of early growth response 2 (EGR2) interleukin 6 (IL6), and insulin-like growth factor 1 (IGF1) tended to be up-regulated [14]. IGF1 has been proposed by other authors to be a possible marker in serum samples of DISH patients [16,17].

The third study in the Special Issue is that of Bischof et al. (2021) [18], who focused on cell culture and tissue-specific progenitor cells. They presented a study on the optimization of culture conditions on the so-called nucleus pulposus progenitor cells (NPPC). In this study, primary IVD cells were isolated from human disc tissue with a mild digestion protocol [19,20]. After reaching confluence in monolayer recovery, these mixed IVD cells, mainly from the nucleus pulposus (NP), were then trypsinized and sorted with a surface marker named Tie2 (or CD202b), which stands for angiopoeitin receptor-1. The Tie2+ cells and the Tie2- cells were then further cultured in normoxic and hypoxic (i.e., 2%) conditions in presence of Angiopoetin-1 (Ang-1) or Ang-2 at increasing doses [21]. However, the results of the study [18] did not produce the expected results, namely, that Tie2+ were stimulated and would proliferate faster compared to Tie2− cells. Despite this, it seemed very clear that hypoxia, i.e., at 2% oxygen level, was the most important factor for higher cellular metabolic activity. Their conclusion that hypoxia is beneficial for these NPPC is in agreement with other studies performed by the team of Daisuke Sakai from Tokai University School of Medicine [22,23].

3. Clinical/Radiological Studies in the Second Special Issue

There are two radiological studies published in this Special Issue. Firstly, Landauer and Trieb (2022) [24] provide radiological evidence that the lumbosacral transitional vertebrae (LSTV) are valid as a model for IVD regeneration. They scanned 1482 patients radiologically, and their LSTV were then classified according to Castellvi classification type II–IV [24]. Additionally, magnetic resonance scans (MRIs) were also obtained from selected patients. The authors concluded that the reduced or absent mobility in the LSTV segments led to an overload of the adjacent segments in these patients.

The second study, by Kim et al. (2022) [25], is on the usage of natural language processing (NLP), which is defined as understanding, analyzing, and extracting meaningful information from text (natural language) by computer science [26]. This research targets a highly significant area of research, which is “big data”. It is obvious that artificial intelligence (AI) will be necessary to make full use of all the available clinical data and to help surgeons to take decisions with the assistance of fast data processing. In this approach, the authors tested their NLP pipeline on a balanced sample of 300 X-ray, 300 CT, and 300 MRI reports. When evaluating their NLP model performances, four parameters—recall, precision, accuracy, and a so-called “F1” score (the harmonic mean of precision and recall [27])—were greater than 0.9 for all 23 radiologic findings.

4. A Review on Secretomes of the IVD

Extracellular vesicles (EVs) have long attracted the attention of the regenerative community. The importance of this topic is underlined by a current “wave” of comparable articles that also focus on the usage of EVs to regenerate the IVD [28–31]. This is not altogether surprising as regulatory hurdles toward proving non-toxicity and patient safety have recently been introduced by authorities such as the Federal Drug and food Agency (FDA) and the label of the Conformité Européene (CE) [32]. This applies in particular to cellular applications. Thus, secretomes, or so-called conditioned media, have been the focus of recent IVD-related research [29,33]. The review by Tilotta et al. (2023) provides a valuable insight into the field of EVs and a summary of their characterization [29].

5. Conclusions

Overall, this Special Issue offers a good insight into the heterogeneity of IVD research and the recent findings not only from clinics, but also from biologists and engineers. I hope that this Special Issue will give scientists an overview of this highly translational and applied fast growing research field. There is still yet further research to come to help to find possible "cures" for affected patients. With the recent prognosis by Ferreira et al. (2023) [1] warning of an increase of one-third more LBP patients in the next 50 years, the scientific community is urged to find better treatment options and also especially early diagnostic tools to foresee critical cases to come.

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