Clonal Human Embryonic Progenitor Cell Line Injections Facilitate Intervertebral Disc Repair and Reduce Nerve Root Pain in In Vivo Animal Models

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Introduction: Intervertebral disc (IVD) degeneration involves decreased levels of extracellular matrix components and increased catabolism and activated inflammatory response. The injection of viable cells, particularly mesenchymal stem cells (MSCs), has been shown in animal models and in humans to facilitate tissue repair and treat pain\(^1-3\). However, MSC heterogeneity and limited plasticity may impact anticipated efficacy, while a limited lifespan may affect large-scale clinical availability. Clonal human embryonic progenitor (hEP) cells are committed progenitor clones that minimize the risk for uncontrolled differentiation typically associated with embryonic stem cells (ESCs) or induced pluripotent stem (iPS) cells, while maintaining a predictable phenotype through extensive population doublings. Clonal hEP cell lines with chondrogenic potential have shown potential for broad availability for transplantation and site-specific differentiation with remarkable expansion capability while maintaining their cellular phenotype. We have reported an ex vivo/in vitro whole-organ rabbit culture system in which the injection of selected clonal hEP cell lines facilitated regenerative and anti-inflammatory effects in degenerative IVDs\(^4\). However, the in vivo effect of hEP cell lines remains uncertain. The purpose of the current study is to assess whether the injection of clonal hEP cells affects IVD degeneration in an in vivo rabbit annular puncture model and further investigate whether injection of clonal hEP cells treats nerve root pain in a degenerated disc tissue-induced radiculopathy model in nude rats.

Methods: All experiments were performed with approval by the Animal Care and Use Committee at our institution. **Study 1** Effect of hEP cell injection in a rabbit degenerative IVD model. Annular puncture model: Twenty New Zealand white rabbits, weighing approximately 3.5 kg, were used in this study. The annular puncture disc degeneration model was used as previously described.\(^5\) Cells: Two clonal hEP cell lines, selected from 100 cell lines and confirmed to possess chondrogenic potential (4D20.8 or 7SM0032), and human MSCs (hMSCs) were used. Cell injection: Four weeks after annular puncture, either 4D20.8, 7SM0032 or hMSCs were injected randomly into L2-3 or L4-5 IVDs at a cell density of \(2 \times 10^5\) cells/10 μl saline with a 26G needle. The L3-4 IVD served as the non-punctured control. Radiographic analysis: Normalized %DHI was monitored.\(^5\) Rabbits were sacrificed at 12 weeks post-cell transplantation, and the intact spinal columns were harvested for MRI analysis (T2-weighted sagittal and axial images), qPCR and histologic analyses to determine the degree of degeneration. **MRI:** Ranges of
interest for nucleus pulposus (NP) and annulus fibrosis (AF) were drawn in sagittal T2 weighted images and template-analysis was performed. Both raw and normalized MRI measures were obtained and compared. qPCR: Gene expressions for matrix-related genes (type II collagen and aggrecan), inflammation-associated genes (IL-1 and IL-6), and catabolism-associated genes (MMP3, ADAMTS4) in the NP and AF were analyzed by quantitative real-time PCR using standards. Histology: Harvested IVDs, prepared for paraffin sections and stained by H&E and Safranin-O, were graded for IVD degeneration as previously described5 (Fig.1).

**Study 2:** Effect of hEP cell injection in the nude rat radiculopathy model. Nude rat disc xenograft radiculopathy model: To assess mechanical and thermal allostodynia, 16 Nude Rats (RNU/RNU; BW 250-300 g, eight rats per group) were used for this study. The right L5 dorsal root ganglion (DRG) was exposed by partial laminectomy and facetectomy and NP tissues (NP, 1x1x1 mm) from either saline- or hEP cell-injected (4D20.8) discs described above (study 1) were placed as xenografts. Allodynia were assessed by the von Frey test (Fig. 2).

**Results:** %DHI: Serial %DHI results demonstrated a significant difference between 4D20.8-injected IVDs versus saline-injected controls at 12 weeks post-annular puncture (p<0.05). A similar significant difference was detected at 6, 8, 10 and 12 weeks in 7SM0032-injected IVDs and at 6, 10 and 12 weeks in hMSC-injected IVDs, respectively. Recovery rate: 4D20.8-injected IVDs showed a trend for recovery at 12 weeks post-annular puncture (p=0.06). MRI: MRI data showed that, compared to saline-injected controls, IVDs injected with 7SM0032 cells or hMSCs tended to have higher T2 values and greater NP area. qPCR: Changes in gene expression showed that 4D20.8-injected IVDs have significantly higher upregulation of type II collagen and aggrecan genes and lower IL-1, IL-6, MMP3 and ADAMTS4 gene expression compared to 7SM0032- or hMSC-injected IVDs or saline controls (p<0.05). Histology: Histological grading scores also demonstrated that 4D20.8-injected IVDs had a significantly lower total degeneration score (7.75) compared to IVDs injected with hMSCs (10.75) or 7SM0032 cells (12) or saline-injected controls (11)(Fig. 3, p<0.05). Nude rat disc xenograft radiculopathy model: In the neuropathic pain model induced by xenografts of NP from the rabbit annular puncture model to the rat DRG, the threshold for responses induced by tactile stimulation with von Frey filaments was significantly decreased after 4 days post-xenograft of the saline-injected NP group, indicating induction of mechanical allodynia that gradually improved over 21 days. Xenografts of the 4D20.8-injected NP group showed significantly higher thresholds at 7 and 11 days post-xenograft and a milder decrease throughout, suggesting an inhibition of mechanical allodynia in the neuropathic pain model (Fig. 4).

**Discussion:** There has been an increasing interest in stem cell therapy to treat degenerative IVD disease. However, the selection of an optimal cell source remains elusive. Many studies have explored the potential of MSCs in various animal models and in humans. Despite the increasing number of studies on MSC injections, for clinical use, MSCs may possess disadvantages, compared to clonal cells, of being heterogenic in character and lacking consistency. For the first time, the results of the current study showed that injection of the clonal hEP cell line 4D20.8 can decelerate the degenerate process detected by radiography, gene expression and histology in an in vivo rabbit annular puncture model, and furthermore reduce allodynia in the nude rat disc xenograft radiculopathy model. These cell lines may provide strong candidates as effective donor cells in cell therapy for IVD disease.

**Significance:** Clonal human embryonic progenitor cells proved to be effective in facilitating IVD regeneration in an in vivo rabbit annular puncture model of IVD degeneration and to reduce pain in the
nude rat disc xenograft radiculopathy model. This effect, similar to that of MSCs, with the ease of access and stabilized quality of these cells, offers advantages over other donor cell types for intradiscal injection therapy.