Characterization of a Novel Progenitor Cell with Therapeutic Potential Derived from Adult Human Intervertebral Disc Tissue

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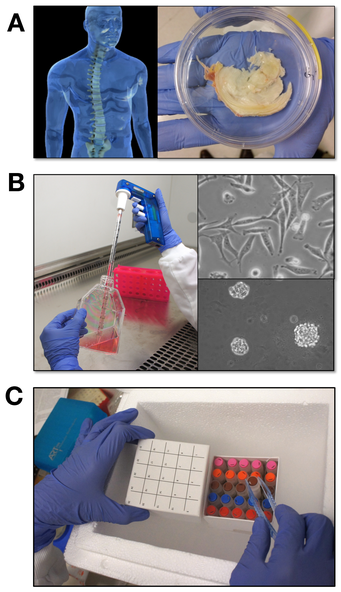
Introduction

- We have developed an *in vitro* process to generate progenitor cells from differentiated nucleus pulposus cells derived from the intervertebral disc, which we call discogenic cells (**Figure 1**).
- These cells have potential therapeutic utility in treating disc disease, as demonstrated by multiple preclinical studies in rabbits and pigs where new extracellular matrix was formed *in vivo*.
- The matrix-forming potential of the cells have been explored previously through histology and PCR (**Figure 2**).
- In this study, the stem properties of these plastic-adherent derived novel cells are characterized in order to better understand potential therapeutic mechanisms of action *in vivo*.

Figure 1: Process for creating discogenic cells. (A) Procure adult disc tissue. (B) Produce discogenic cells through proprietary multi-step process. (C) Create frozen bank of discogenic cells; release testing to ensure safety and consistency.



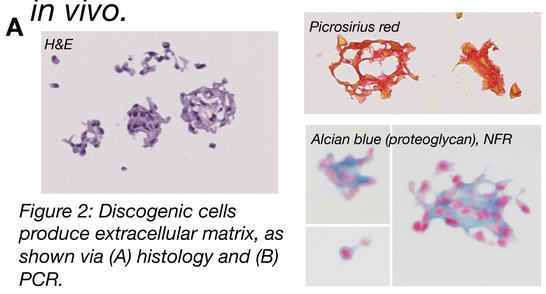


Figure 2: Discogenic cells produce extracellular matrix, as shown via (A) histology and (B) PCR.

Methods

- Discogenic cells were cultured in a pro-osteogenic, pro-chondrogenic and pro-adipogenic environment to assess multipotentiality. Three lots assessed, representative data from 1 lot shown.
- Expression of surface antigens classically associated with stemness (positive for CD105, CD73, CD90; negative for CD34, HLA-DR) was measured. Additional surface markers Stro-1 and CD271 were assessed.
- RT-PCR was used to assess expression of NANOG, Oct ¾, and Sox9 compared to early-process cells for 5 different lots of discogenic cells and MSCs, normalized to housekeeping gene.

Results

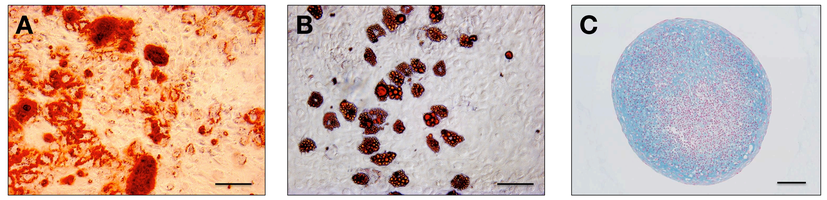
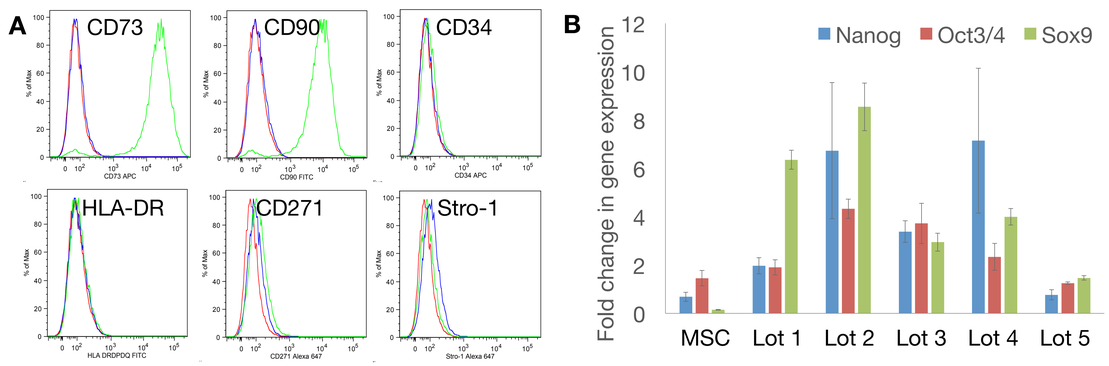


Figure 3: Cells were differentiated into (A) osteogenic, (B) adipogenic and (C) osteogenic lineages.

- Discogenic cells were successfully differentiated into the three lineage cell types, as evidenced by positive staining for hydroxyapatite, lipids and proteoglycan in each condition (**Figure 3**).
- Flow cytometry demonstrated positive (>85%) expression for CD73, CD90 and negative (<3%) expression for CD34 and HLA-DR. Also, the discogenic cells were negative (<10%) for Stro-1 and CD271 (**Figure 4A**).
- PCR showed an upregulation of stemness markers (**Figure 4B**).

Figure 4: (A) Flow cytometry histograms (green – stained; blue – isotype control; red – unstained control). (B) Gene expression profile of 5 lots for stemness markers.



Conclusions

- Discogenic cells satisfy key criteria for stemness, including plastic adherence, expression of certain surface markers, and multipotentiality. Further, PCR shows expression of genes that are associated with stem cells.
- Given that the discogenic cells also function as committed cells by producing extracellular matrix native to the nucleus pulposus, we define these novel cells as progenitors.
- We now plan to examine additional *in vivo* mechanisms of action related to stemness, such as anti-inflammatory properties and paracrine effects. Human trials are anticipated.

This product is under preclinical investigation and has not been approved for use by the FDA.

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