Evaluation of Intervertebral Disc-Derived Progenitor Cells (Discogenic Cells) Implanted into Subcutaneous Pouches of Athymic Mice

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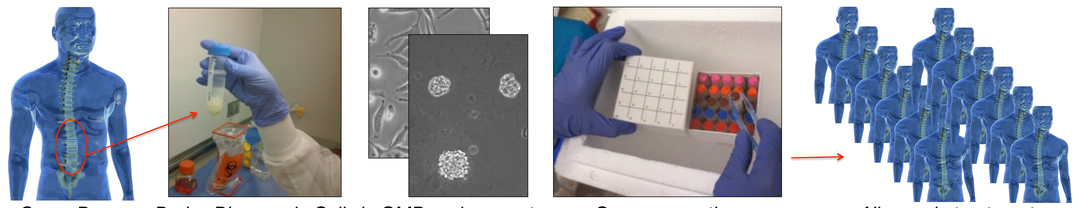
Introduction

- Our group has developed a method to generate discogenic cells, which are progenitor cells derived from adult intervertebral disc tissue.
- These cells are intended to treat moderate degenerative disc disease.
- Previously, we showed that the cells restore disc height and tissue architecture in injured rabbit and pig discs (ORS 2014, 2015, 2016, 2017).
- Here, we inject the cells into subcutaneous pouches of athymic mice to evaluate tumor formation and regenerative capacity. Also, tumorigenic cells were injected as positive control. This model is recognized by FDA to evaluate tumor formation potential.
- Clinical-grade cells were used in this study, in order to be able to translate the findings to anticipated risks and benefits in a Phase 1 human clinical trial.

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



Methods

- This study was approved by a private IACUC. This study adhered to GLP standards.
- Male and female athymic mice were injected with either a supra-physiological dose of discogenic cells (10 million per 0.2 mL) with sodium hyaluronate and cryopreservatives, a cell-free injection of vehicle alone, or 10 million HeLa cells in EMEM into a subscapular pouch (n=10 animals/group).
- Animals were observed daily for body weight, morbidity, mortality, and for the presence of tumors by palpation. When tumors were found with a diameter greater than 20 mm in any dimension, the animal was sacrificed.
- After 4 months, the animals were euthanized and a gross necropsy was performed. The injection site, any lesions, and a selection of organs and tissues were collected for histological processing (H&E staining) and blinded evaluation by a board-certified veterinary pathologist for abnormalities.
- When regions of disc-like tissue were identified, the sections of tissue were additionally stained with safranin O and picrosirius red. Immunohistochemical techniques were utilized to stain for nuclear mitotic apparatus protein (NuMa) to identify human cells, and Ki-67 to identify proliferating cells.
- Differences in body weight between groups at each timepoint were compared using a 1-way ANOVA and post-hoc tests.

Results

- Body weight increased over the course of the study for all animals (Figure 1A). All animals in cells+vehicle group survived until the scheduled termination, whereas most of the HeLa-dosed animals were terminated early (Figure 1B). All HeLa groups had palpable nodules. After an initial appearance of nodules that subsided, no cells+vehicle or vehicle animals had nodules at 4 months (Figure 1C). Nodule size increased over time for the HeLe group (Figure 1D).

Results

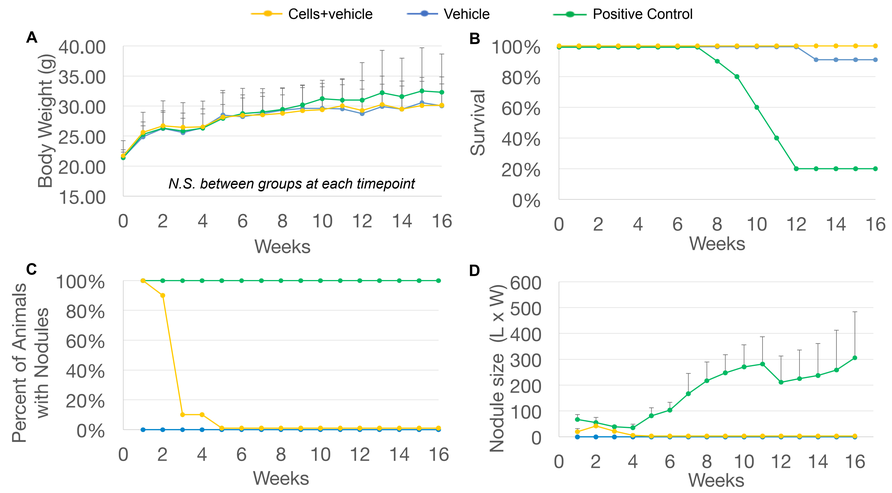
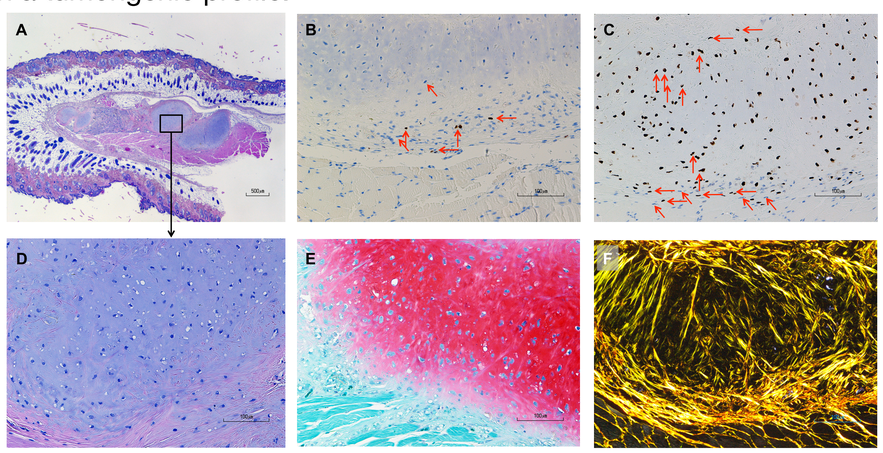


Figure 2: (A) Body weight (B) survival, (C) percent of animals with palpable nodules and (D) average nodule size (error bar shows standard deviation).

- In 4 of 10 animals that received cells+vehicle, regions of cartilaginous, disc-like tissue were identified. These masses were small in size, encapsulated, and contained well-developed, non-neoplastic cartilage, fibrocartilage and dense fibrous connective tissue (Figure 3A) containing human discogenic cells (Figure 3B). Proliferation, indicated by Ki-67, was present at low levels (Figure 3C). The cartilaginous matrix produced by cells+vehicle consisted of proteoglycan mixed with collagen fibers of varying sizes (Figure 3D, E, F)).
- The other tissues and organs evaluated in animals treated with cells+vehicle did not contain neoplasms or distal metastases, consistent with a non-tumorigenic phenotype. When evaluating the animals that were treated with vehicle, no patterns of histologic findings were noted. In contrast, all animals that received the positive control (HeLa cells) developed carcinomas at the injection site, consistent with a tumorigenic profile.

Figure 3: A) H&E of encapsulated cartilage-like tissue. B) NuMA (human cells) and C) K-67 (proliferating cells). For both, vertical arrow indicates chondrocyte-like cells, horizontal arrow indicates fibroblast-like cells and diagonal arrow indicates host cells. D) Higher magnification H&E. E) Safranin O stain. F) Picrosirius red stain under polarized light.



Conclusions

- Taken together, these studies demonstrate that discogenic cells and the proposed carrier material do not exhibit any notable safety concerns when injected into subcutaneous pouches of athymic mice, including not forming tumors or causing abnormalities in distal organs/tissues.
- Further, the cells generate intervertebral-like tissue in some instances, demonstrating the regenerative capacity of the progenitor cells and supporting the proposed mechanism of action for the treatment.
- When considered in conjunction with animal studies where the cells are applied directly to the disc, such studies demonstrate safety and bioactivity, supporting that human clinical trials are warranted.

This product is being evaluated in a clinical study and is not yet approved for commercial human use.

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