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Autologous Mesenchymal Stem Cell Transplantation in Male Stress Urinary Incontinence

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BACKGROUND

Radical prostatectomy is the most traditional treatment for prostate cancer, which affects one out of every seven men. One of the most common side effects of a radical prostatectomy is stress urinary incontinence. [1] A 2010 study states that:

At 6 weeks after surgery 59% (405) of men were incontinent, defined as any pad use. At 58 weeks after surgery 22% (165) of men were incontinent. At 58 weeks incontinence was more prevalent in men who were obese and physically inactive (59% incontinent). Physical activity may offset some of the negative consequences of being obese because the prevalence of incontinence at 58 weeks was similar in the obese and active (25% incontinent), and nonbese and inactive (24% incontinent) men. The best outcomes were in men who were nonobese and physically active (16% incontinent). Men who were not obese and were active were 26% less likely to be incontinent than men who were obese and inactive (RR 0.74, 95% CI 0.52–1.06). [2]

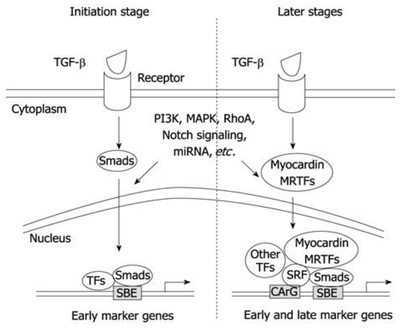
Furthermore, almost 10% of patients experience urinary incontinence permanently. Conservative treatment for stress urinary incontinence in men consists of pelvic floor exercises. In cases when conservative treatment is unsuccessful, “urethral bulking agents, male slings, and the artificial urinary sphincter” can be used. Stem cells have proven to have regenerative effects and have been looked into as a treatment for urinary incontinence in women, by using stem cells to repopulate missing smooth muscle in the pelvic floor muscles. [3] Therefore, it is necessary to look into stem cells as a possible treatment for male urinary incontinence.

An animal trial is a necessary step in accessing how safe an effective a possible drug can be. Generally, it is difficult to find a mouse model that displays incontinence. Previous animal models used to test stem cell treatments for urinary incontinence involved artificially creating incontinence in mice.[3] In order to determine if a mouse is urinary incontinent, one can observe the urination pattern of a mouse model in a cage. Generally, mice urinate in one or two discriminate locations, which can be observed by placing filter paper on the bottom of the cage. When a mouse is urinary incontinent, there would be spots on the filter paper indiscriminately. [4] There is an animal model that is currently being used in a bladder cancer study associated with New York Medical College and other institutions that display the indiscriminant urination pattern, and have been determined to be an excellent model of urinary incontinence. The goal of the summer was to determine how to consistently produce smooth muscle cells from mouse bone marrow.

Following review of literature, Transforming Growth Factor Beta (TGF-ß) was decided to be the cytokine of choice. It has been proven that TGF-ß can cause smooth muscle cell differentiation through the SMAD2/SMAD3 pathway and its role in increasing the expression of myocardin, another factor in smooth muscle differentiation. Both TGFß-1 and TGFß-3 were investigated throughout the study. Finally, the projected to attempted to find the ideal conditions for smooth muscle differentiation using the TGF-ß pathway.

HYPOTHESIS

Mesenchymal stem cells may be useful in the treatment of post prostatectomy stress urinary incontinence by populating sphincteric scar with viable smooth muscle cells.

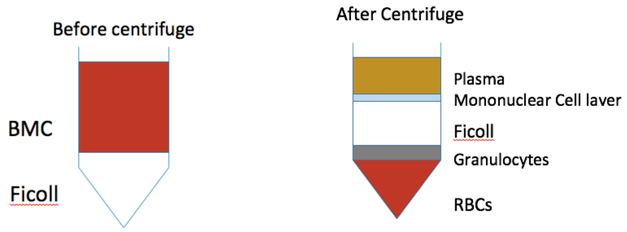


The following figure demonstrates the process by which TGF-ß can stimulate smooth muscle cell differentiation. TGF-ß can both directly stimulate smooth muscle cell differentiation and upregulates the expression if myocardin which assists in the differentiation of smooth muscle cells.

METHODS

Bone Marrow Derivation.

Animal protocols were approved by the Institutional Animal Care and Use Committee at New York Medical College. Standard protocol was followed to remove the bone marrow from the mice. [5] Following collection of bone marrow, a Ficoll Paque gradient was used to separate the cells in bone marrow by density. The mesenchymal stem cells were collected from the gradient and resuspended in Iscoves Modified Dublecco’s Media (IMDM) with 20% FBS at a concentration of 10^6 cell per mL of serum in petri dishes. The cells were cultured for three weeks and were fed every three days.



Treatment

Following aspiration of IMDM/20% FBS from one 60 mL dish of MSCs, cells were resuspended with trypsin/EDTA. Cells were then spun at 1000 RPM, 31˚C for 10 minutes, and the supernatant was removed. The remaining pellet was placed in 4 mL of IMDM/20% FBS. 2 mL of the serum mixed with cells were placed in one column (4 chambers) of a Lab-Tek chamber slide. TGFß-1/TGFß-3 was then added at a concentration of 1 ng/mL. The remaining mix was then added to the other column in the Lab-Tek chamber slide. Allow the chamber to incubate at 4 ˚C for 72-hours.

Shown is an example of a Lab-Tek Chamber slide. The unique slide contains eight wells that can be removed after testing to allow a quick transfer from testing to analysis.

IMDM/20% FBS/TGFß-3 serum was removed from the chambers by aspiration. The chambers were then washed with phosphate buffer saline (PBS), and then filled with chilled menthol. After a 15-minute incubation period, the chambers three times using PBS. The cells were then put in block and allowed to incubate for 24-hours. Following the incubation period, select antibodies (calponin, desmin, smooth muscle actin, and cytokeratin) were diluted in block according to manufacturer recommendations. The antibodies were added to the chambers, each row (2 chambers) receiving a different antibody. Following a second 24-hour incubation period, appropriate secondary antibodies were diluted in block according to manufacturer recommendations. The secondary antibodies were added to the appropriate chambers and allowed to incubate for two hours. DAPI diluted in PBS was then added to the chamber, and allowed to incubate for five minutes. Following incubation, the chambers were removed and the slide was mounted using a lab made glycerol based solution. Slide were investigated under a microscope and pictures were acquired using a digital camera and a confocal microscope.

RESULTS

Results:

Immunostaining was used to test the presence of smooth muscle differentiation. By observing the characteristics expressed, one could determine if the mesenchymal stem cells had been differentiated into smooth muscle cells. An example of immunostains seen in a differentiated colony of smooth muscle cells and a nondifferentiated colony of smooth muscle cells are shown in figure 1. Following the experiment, TGFß-1 failed to cause smooth muscle differentiation in the mesenchymal stem cell. The TGFß-3 treatment resulted in a positive differentiation of smooth muscle cells from mesenchymal stem cells. The left image in figure 1 shows the presence of smooth muscle actin in cells treated with TGFß-3. Figure 2 shows the presence of calponin, another smooth muscle marker, in a different colony of cells treated by TGFß-3. Following the results, a second experiment was conducted in order to determine the ideal exposure for the treatment of MSCs with TGFß-3. The methods above were followed until the treatment time for the cells. Half of the chambers were treated for 24-hours and half the chambers were treated for 48 hours. A representation can be seen in figure 3.

RESULTS CONTINUED

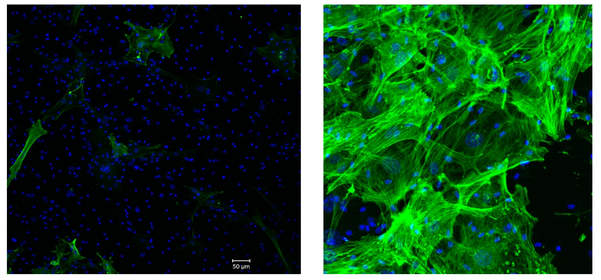


Figure 1. Negative and Positive Immunoassay for Smooth Muscle Charactersitics. The images above were captured by a confocal miscroscope. The blue are DapI stained nuclei, while the green are FITC marked smooth muscle actin, a characteristic found in smooth muscle cells. The image on the right shows a colony that did not differentiate, while the image on the right shows a colony that did.

Figure 2. Calponin in TGFß-3 treated cells. The image was captured using a confocal microscope. The blue DapI stains are the nuclei and the green FITC stain are antibodies for calponin. The Image above shows presence of calponin in the treated cells.

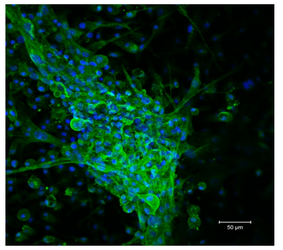
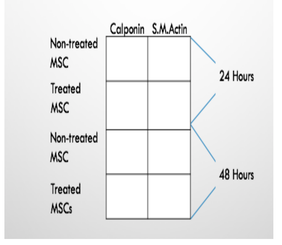


Figure 3. Time course of TGFß-3 treatment. The boxes represent the eight chambers of a Lab-Tek chamber slide. non-treated MSCs and treat MSCS were placed in alternating rows. The top half of the chambers were only treated for 24-hours while the bottom half of the chambers were treated for 48-hours. One column was then stained for calponin while the other column was stained for smooth muscle actin.



This second experiment showed that the MSCs that were treated for 48-hours showed more smooth muscle characteristics than the MSCs that were treated for 24 hours. Due to an error with DAPI staining, the exact ratio of cells that successfully differentiated to smooth muscle cells could not be determined, but it appeared that the ratio of differentiation in the 48-hour treatment is similar to the 72-hour treatment.

CONCLUSIONS

The study found that while TGFß-1 is not an optimal cytokine to consistently produce smooth muscle cells, TGFß-3 is. Additionally, it was determined that though the previous study that used TGFß-3 treated the cells for only 24-hours, it is more optimal to treat the cells for 48-hours or 72-hours. The next step of the project is to inoculate the smooth muscles into the urinary incontinent mouse model and see the results.

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