Autologous Mesenchymal Stem Cell Transplantation in Male Stress Urinary Incontinence

Benjamin Mann  
*New York Medical College*

Keerat Kaur  
*New York Medical College*

John Phillips  
*New York Medical College*

Carol Eisenberg  
*New York Medical College, carol_eisenberg@nymc.edu*

Leonard M. Eisenberg  
*New York Medical College, leonard_eisenberg@nymc.edu*

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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% inactive). Physical activity may offset some of the negative consequences of being obese because the prevalence of incontinence at 58 weeks was similar in obese and active (25%), and nonobese and inactive (24%) patients. The best outcomes were in men who were nonobese and physically active (16% inactive). 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 includes pelvic floor exercises. In cases where conservative treatment is unsuccessful, "urinary 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, 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. [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.

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-β) was decided to be the cytokine of choice. It has been proven that TGF-β can cause smooth muscle cell differentiation through the SMAD2/3/4/7 pathway and its role in increasing the expression of myocardin, another factor in smooth muscle differentiation. Both TGFβ-1 and TGFβ-3 were investigated throughout the study. Finally, the project was attempted to find the ideal conditions for smooth muscle differentiation using the TGFβ-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.

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 other column was stained for the cytokine of choice. It has been proven that TGF-β can cause smooth muscle cell differentiation through the SMAD2/3/4/7 pathway and its role in increasing the expression of myocardin, another factor in smooth muscle differentiation. Both TGFβ-1 and TGFβ-3 were investigated throughout the study. Finally, the project was attempted to find the ideal conditions for smooth muscle differentiation using the TGFβ-pathway.

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 Iscove Modified Dulbecco’s Media (IMDM) with 20% FBS at a concentration of 10^6 cells per mL of serum in petri dishes. The cells were cultured for three weeks and fed every three days.

RESULTS CONTINUED

![Image](image-url)

Figure 1. Negative and Positive Immunostains for Smooth Muscle Characteristics. The images above were captured using a confocal microscope. The blue DapI stains the nuclei and the green FITC stain are antibodies for calponin. The Image shows presence of calponin in the treated cells.

![Image](image-url)

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

![Image](image-url)

Figure 3. Time course of TGFβ-3 treatment. The boxes represent the eight chambers of a Lab-Tek chamber slide, non-treated MSCs and treated 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 MSC’s that were treated for 48-hours showed more smooth muscle characteristics than the MSC’s 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 TGFβ-1 is not an optimal cytokine to consistently produce smooth muscle cells, TGFβ-3 is. Additionally, it was determined that though the previous study that used TGFβ-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.

BIBLIOGRAPHY