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% (365) 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 nonobese 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 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 assessing 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 urinary pattern of a mouse 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 indiscernible. [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 myosin, another factor in smooth muscle differentiation. Both TGF-1 and TGF-ß3 were investigated throughout the study. Finally, the project was attempted to find the ideal conditions for smooth muscle differentiation using the TGF-ß pathway.

Mesenchymal stem cells may be useful in the treatment of post prostatectomy stress urinary incontinence by populating sphincteric scar with viable 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 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 were fed every three days.

### RESULTS CONTINUED

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 columns in the Lab-Tek chamber slide. Allow the chamber to incubate at 4˚C for 72 hours.

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 3 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 MSC’s 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

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 incontinence mouse model and see the results.

### CONCLUSIONS

BIBLIOGRAPHY