Alterations in the Cellular Composition of the Mouse Bladder Following Ovariectomy, Partial Bladder Outlet Obstruction, and Aging

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Alterations in the Cellular Composition of the Mouse Bladder Following Ovariectomy, Partial Bladder Outlet Obstruction, and Aging

Diane H. Smith

A Thesis Submitted in Partial Fulfillment of the Honors Program Requirements at the University of Connecticut

May 2010

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ABSTRACT

Detrusor underactivity (DU) increases susceptibility to urinary retention and accordingly further complicates the management of urinary incontinence. Bladder muscle stretch, a lack of estrogen, and aging are 3 notable DU risk factors. The aim of this research is to better characterize the changes in cellular composition of the bladder that result from these 3 risk factors to gain a better understanding of DU pathogenesis and pathobiology. This research focuses on the effects of a lack of estrogen while also providing an outline for determining the effects of bladder muscle stretch and aging on the cellular composition of the bladder.

5 transgenic mice underwent bilateral ovariectomy and 2 sham control animals were established. The mice exhibited endogenous fluorescence for collagen 3.6 and alpha smooth muscle actin (α-SMA) and antibodies were used to detect vimentin and calponin. 1 week post surgery, decreased expression of α-SMA and calponin revealed a loss of smooth muscle and a more pronounced decrease in calponin expression compared to α-SMA expression suggested that following ovariectomy the smooth muscle transitions to a less mature smooth muscle cell (SMC) phenotype.

Further studies are needed to better characterize both the endogenous fluorescence in transgenic mice and the changes in cellular composition of the bladder in DU pathogenesis and pathobiology.
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INTRODUCTION

The detrusor muscle is made of smooth muscle and constitutes a layer of the urinary bladder wall. Detrusor underactivity (DU) is defined as, “a contraction of reduced strength and/or duration, resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying within a normal time span” [1]. DU increases susceptibility to urinary retention and accordingly further complicates the management of urinary incontinence. Like urinary incontinence, DU is especially common in geriatric populations. Moreover, although other risk factors exist, bladder muscle stretch, a lack of estrogen, and aging are 3 notable risk factors and their effects interact through a common pathway.

Useful animal models exist for all 3 of these notable DU risk factors. Partial bladder outlet obstruction (pBOO) surgery has been used as an animal model for DU in mice with the characteristic symptoms of sarcopenia and fibrosis being reproducibly achieved 3 weeks following surgery [1]. Similarly, bilateral ovariectomy provides a useful animal model for a lack of estrogen. When using animal models to investigate aging, it is important to include multiple age groups that are neither too young nor too old [2]. Furthermore, care must be taken to ensure and provide evidence that aged animals are pathogen free.

The aim of this research is to characterize the changes in the cellular composition of the mouse bladder that result from DU by examining the changes in cellular composition due to muscle stretch, lack of estrogen, and aging. Although smooth muscle predominates, the bladder also comprises other tissue types, including epithelial, nervous, macrophages, and connective. DU is characterized by changes in these tissues involving bladder muscle loss (sarcopenia), fibrosis, and axonal degeneration [3].
Many cell types retain some level of plasticity, allowing them to modify their phenotypes in response to environmental changes and genetic programs. SMCs exhibit significant plasticity, allowing them to both quickly and reversibly change phenotype in response to environmental cues [4]. Different SMC phenotypes correspond to different SMC functions throughout development, tissue repair, and disease [4]. Additionally, a unique set of marker proteins are characteristic of each phenotypic state. The expression of marker proteins in SMC differentiation and maturation can be considered along a continuum in which marker proteins of less differentiated SMC are first expressed in earlier developmental stages while marker proteins of more mature SMCs are expressed in later stages of SMC development. Although this continuum of SMC development was traditionally interpreted as signifying that all mature SMCs developed from the least differentiated SMCs, a recent study suggests that SMC precursors give rise to naïve SMCs, which have a synthetic phenotype, but then give rise to a variety of SMC phenotypes along the continuum [5]. As in the earlier perspective of SMC development, this new model also recognizes the high degree of plasticity exhibited by SMCs. Furthermore, investigations of the SMC phenotypic diversity recognized contractile and synthetic SMCs as being at opposite ends of the SMC phenotypic spectrum [5]. Contractile SMCs are elongated, spindle-shaped, and contain many contractile filaments [5]. Synthetic SMCs are less elongated, contain many organelles needed for protein synthesis, and have higher growth rates as well as greater migratory activity. Contractile and synthetic SMCs, as well as the various intermediate SMC phenotypes, can be distinguished with marker proteins.

In the presented research, changes in the smooth muscle of the mouse bladder due to bladder muscle stretch and a lack of estrogen are monitored by observing changes in the relative numbers of cells expressing α-SMA and calponin following pBOO or bilateral ovariectomy.
αSMA is a commonly used marker protein for less differentiated SMCs whereas calponin is expressed in more mature SMCs [4]. Following one of the prescribed treatments, an increase in the number of SMCs expressing αSMA and a decrease in those expressing calponin would indicate that the smooth muscle tissue became less differentiated, towards a synthetic phenotype, which characterizes sarcopenia and would be expected following bladder muscle stretch, a lack of estrogen, or aging.

Similar to SMCs, fibroblasts also exhibit significant plasticity and are monitored in this research by observing the changes in the relative numbers of cells expressing 2 marker proteins, collagen 3.6 and vimentin. Increases in both marker proteins are characteristic of fibrosis and therefore expected following bladder muscle stretch, a lack of estrogen, or aging. Figure 1 provides a summary of the expected changes in all 4 examined marker proteins following each treatment.

**Figure 1. Proposed Changes in Bladder Composition with Injury.**
Studies suggest that following partial bladder outlet obstruction (pBOO), there is initially a period of hypertrophy preceding the muscle atrophy and fibrosis that is observed in longer-term studies.
This research is important as it could increase our limited understanding of DU pathophysiology and pathogenesis, which will allow for the development of more effective, rather than simply palliative, treatments for urinary incontinence. Currently, urinary incontinence in the frail elderly is treated mostly with behavioral therapies while research on treatment options remains limited [6]. On a broader scale and by considering the general simplicity of the bladder, this research may contribute to the understanding of other geriatric problems by serving as a model for a better understanding of both pathogenesis and treatment in more complex tissues.

MATERIALS AND METHODS

Animal care. Double transgenic mice with fluorescent markers for αSMA (mcherry, red) and 3.6 collagen (cyan, blue) were obtained from the lab of Dr. Ivo Kalajzic at the University of Connecticut Health Center. These mice were then bred and maintained in accordance with the National Institute of Health guidelines. A local animal care committee approved all procedures and the mice were allowed ad libitum access to food and water.

Animal surgeries. All surgeries were performed on 5 wk old female mice. 5 mice underwent bilateral ovariectomies. 7 mice underwent partial bladder outlet obstruction (pBOO) as previously described [1], of which 2 died after surgery. Following both surgeries, mice were sacrificed 1 week post surgery. Similarly, sham controls were established for both surgeries, 2 for ovariectomies and 4 for pBOO.

Tissue collection and dissociation. Mice were euthanized with isofluorane. After rinsing the abdomens with ethanol, the bladders were immediately extracted, blotted dry, and placed in cold PBS solution. In sterile conditions, the bladders were finely minced with a scalpel and razor
blade and then introduced to filtered dissociation buffer (collagenase P in PBS with a final concentration of 1 mg/mL passed through a 0.2 μm filter). Following trituration through the fire-polished tip of a Pasteur pipette, the samples were placed in a 37°C shaker for 45 min. An equal volume of trypsin was then added to each sample and the reaction was allowed to continue for 5 min in a 37°C water bath. During this time, the samples were shaken twice. The reactions were stopped by adding 10% FBS. Next, samples were passed through a 70 μm filter and centrifuged at 1500 rpm for 5 min. The obtained pellet was re-suspended in 8 mL of wash buffer (10% FBS in PBS) and centrifuged as before. The pellet was re-suspended in 1 mL of medium (DMEM/F12 with 10% FBS+P/S, 50x dilution). Cell count was then ascertained using a drop of this suspension and Trypan blue under 10x magnification.

**Sample preparation for FACS analysis.** Samples were prepared for FACS analysis in one of 2 ways, depending on whether or not antibodies were incorporated into the sample analysis. The first method, for fluorescent samples for which antibodies were not incorporated, involved first washing the cells 2 times with FACS buffer (into a 1.5 mL tube and adding 1.2 mL FACS buffer). The samples were then centrifuged at 1300 rpm for 5 min at 4°C. 500 μL of FACS buffer was added to the obtained pellets and after mixing by vortexing, the samples were ready for FACS analysis.

The second FACS preparation method, for samples analyzed with antibodies, involved first following the above procedure for samples not incorporating analysis with antibodies. These samples were then fixed by adding 150 μL fixing buffer, vortexing, and waiting 30 min. The antibodies were then introduced as described below. Next, the samples were washed with 500 μL FACS buffer, centrifuged as before, and the obtained pellet mixed with 500 μL FACS...
buffer via vortexing. Unlike the unfixed samples, these samples could be stored at 4°C and did not need to immediately undergo FACS analysis.

**Immunohistochemistry.** Monoclonal antibodies against vimentin and calponin were obtained from Abcam (ab8978) and Sigma (C2687) respectively. Monoclonal antibody labeling kits were obtained from Invitrogen and used to conjugate vimentin and calponin to the fluorophores Alexa Fluor 647 and Pacific Blue respectively. The conjugated antibodies were introduced to the fixed cells by first permeabilizing the cells with permeabilization buffer (eBioscience) and then incubating the permeabilized cells on ice with the antibodies for 25 min.

**Tissue characterization.** FACS analysis was used to analyze all samples. The efficacy of the transgenic markers cherry and cyan, for αSMA and collagen 3.6 respectively, was tested by comparing the cherry and cyan signals for wild type, cyan only, cherry only, and double transgenic mice. This analysis was performed using the FACSARia machine.

The efficacy of staining with fluorophore conjugated antibodies against vimentin and calponin was assayed by comparing the Alexa Fluor and Pacific Blue signals for cell samples treated with the antibodies to unstained cells. This analysis was performed using the LSR II machine.

The changes in bladder cellular composition following ovariectomy and pBOO were investigated by comparing the signals from the 4 markers for αSMA, collagen 3.6, vimentin, and calponin, between the mice that had undergone ovariectomy or pBOO and their corresponding sham controls. All mice were double transgenic and the FACSARia machine was used.

**RESULTS**
The fluorescence of both cyan and cherry in the untreated double transgenic mouse was comparable to the corresponding fluorescence in each of the single transgenic controls, showing that neither signal is disrupted in the double transgenic control (Figure 2). For example, $16.1 + 0.51 = 16.61\%$ of cells in the cyan only control expressed cyan whereas $6.42 + 9.18 = 15.6\%$ of cells in the double transgenic mouse expressed cyan. Additionally, $9.18\%$ of cells in the double transgenic bladder expressed both cyan and cherry and are therefore considered myofibroblasts.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wild Type</th>
<th>Cherry Only</th>
<th>Cyan Only</th>
<th>Double Transgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACSAria Analysis</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 2. Efficacy of Cherry and Cyan Markers**
This data was obtained from the FACSAria machine and shows that cherry and cyan are suitable markers for α-SM actin and collagenase 3.6 respectively in the double transgenic mouse model.

Following cell fixation, nearly a 50% reduction in the endogenous fluorescence of the transgenic fluorophores was obtained. This is evident in Figure 3, which shows cherry expression in the double transgenic mouse before fixing, $9.18 + 32 = 41.18\%$, and after fixing, 21%.

<table>
<thead>
<tr>
<th>Tissue Preparation</th>
<th>Not Fixed</th>
<th>Fixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>No Treatment</td>
<td>Sham Control for OVx</td>
</tr>
</tbody>
</table>

| FACSAria Analysis | ![Image](image5.png) | ![Image](image6.png) |

**Figure 3. Changes in Cherry Fluorescence when Cells are Fixed**
The cherry signal of 2 double transgenic mice is compared and shows that fixing the cells reduces the endogenous cherry fluorescence by approximately 50%, from 41.18% to 21%. SSC-A refers to side scatter.
Cells from double transgenic mice were stained with antibodies against vimentin and calponin. The anti-calponin antibody is conjugated to the Pacific Blue fluorophore. In Figure 4, the no staining sample shows that there is a small amount of overlap between the signals for collagen 3.6 (cyan) and calponin, which is why the Pacific Blue label is retained throughout the figure. However, the overlap is only 1.75% and accordingly is not considered significant. Overall, it is evident that 1 week post ovariectomy, the percentage of cells stained for calponin decreases, $5.13 + 50.2 = 55.23\%$ compared to $7.25 + 60.3 = 67.55\%$. A small decrease is also observed in vimentin, $10.6 + 50.2 = 60.8\%$ compared to $4.36 + 60.3 = 64.66\%$.

<table>
<thead>
<tr>
<th>Staining</th>
<th>No Staining</th>
<th>Double Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>OVx (1 wk)</td>
<td>Sham Control</td>
</tr>
<tr>
<td>FACS Analysis</td>
<td></td>
<td>OVx (1 wk)</td>
</tr>
</tbody>
</table>

![Graphical representation of staining](image)

**Figure 4. Double Staining and Cellular Composition Changes in the Bladder with Ovariectomy**

1 week post ovariectomy, there is a significant decrease in calponin expression and slight decrease in vimentin expression.

Due to the overlap between the cyan and Pacific Blue, the fluorophores for collagen 3.6 and calponin respectively, the changes in cyan expression 1 week post ovariectomy could be not ascertained. Given the relatively minor contribution of endogenous cyan to the Pacific Blue signal (Figure 4: No Staining), the overlap is not considered to significantly impact the calponin expression as determined by the Pacific Blue signal. The changes in αSMA, calponin, and vimentin expression following ovariectomy can therefore be ascertained and are illustrated in
Figure 5. 1 week post ovariectomy, significant decreases in expression are observed in αSMA (14.7% compared to 21%) and calponin (52.9% + 29.9% = 82.8% compared to 51.7% + 40.2% = 91.9%) while a slight increase is observed in vimentin expression (1.78% + 52.9% = 54.68% compared to 0.36% + 51.7% = 52.06%)

Similar changes in protein expression, that being decreases in αSMA and calponin expression accompanied by an increase in vimentin expression, are expected to also occur with pBOO.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fluorescence</th>
<th>Endogenous Cherry</th>
<th>Intracellular Staining for Vimentin and Calponin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.36</td>
<td>51.7</td>
</tr>
<tr>
<td>OVx (1 wk)</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.76</td>
<td>52.9</td>
</tr>
</tbody>
</table>

Figure 5. Changes in Cellular Composition of the Bladder with Ovariectomy
Overall, 1 week following ovariectomy, decreases in cherry fluorescence (corresponding to αSMA expression) and calponin expression are observed. The above Pacific Blue expression is attributed to calponin because the overlap with cyan is insignificant. Vimentin expression slightly increases 1 week post ovariectomy.
DISCUSSION

The presented results demonstrate that 1 week following ovariectomy, there is a significantly decreased expression of $\alpha$SMA and calponin as well as a slight increase in vimentin expression. This corresponds to muscle degeneration and fibrosis, which are both characteristic of DU. These findings confirm that bilateral ovariectomy provides a useful model of DU, even 1 week following surgery.

In the double transgenic, untreated animal, 9.18% of the cells fluoresced both cyan and cherry. These cells may represent myofibroblasts although the percentage of myofibroblasts in the healthy bladder was not expected to be this high. Using a pBOO model, Bouro et al. proposed that following bladder injury, serosal thickening is achieved in a 2 step process in which mesenchymal cells adopt a myofibroblast phenotype first and then transition into a fetal SMC phenotype [7]. Therefore, in the injured bladder, myofibroblasts play a crucial role in tissue remodeling and may be more abundant following bladder injury.

The purpose of this research is to better characterize the pathogenesis and pathobiology of DU and it was found that following ovariectomy, there is a general trend towards less differentiated SMC. This dedifferentiation is evident in that calponin, which is a more mature SMC marker than $\alpha$-SM actin, exhibited a more pronounced decrease in expression than $\alpha$SMA 1 week after ovariectomy (Calponin expression decreased $91.9 - 82.8 = 9.1\%$ whereas $\alpha$SMA expression decreased $21 - 14.7 = 6.3\%$). Similarly, vimentin, which is an early fibroblast marker did not exhibit a significant change in expression.

It is also important to note that, especially with the endogenous fluorescence, the percentage of cells exhibiting fluorescence of a particular fluorophore may not represent the actual percentage of cells expressing the corresponding marker within a sample. This is
especially evident in Figure 3 which shows that fixing cells from double transgenic samples reduces their fluorescence by approximately 50%. Moreover, although αSMA is an earlier SMC marker than calponin and would therefore be expressed in all SMCs that express calponin, only 21% of cells from the Sham control expressed αSMA while 91.9% expressed calponin (Figure 5). Further studies are needed to better characterize the endogenous fluorescence in transgenic mice, as explained in “Future Directions.” Nevertheless, the change in fluorescence of each fluorophore provides valuable information on how the expression of each marker changes.

**CURRENT RESEARCH**

**Additional Considerations**

The presented research investigated how bladder muscle stretch and a lack of estrogen affect the bladder cellular composition. The current research investigates the effects of aging on bladder cellular composition. Although this is a continuation of the previous study, there are several important points and differences to consider. First, although some studies suggest aging contributes to DU [3], a study by Brierly et al. found no correlation between DU and age [8]. In addition to these mixed findings, the possibility of comorbidities further complicates aging studies and care must be taken when selecting which age groups to study. Similarities are evident between developmental and aging processes such that many of the observed physiological changes characteristic of development are thought to occur in the opposite direction during aging. In other words, aging may resemble dedifferentiation. Accordingly, 3 different age groups are examined during this study to include young, old, and intermediate aged mice.
The differences between the current research and the presented research are highlighted in the following section. In addition to a new tissue collection and dissociation protocol, the cell types examined and accordingly also the marker proteins employed present notable differences. For example, in addition to SMCs and fibroblasts, the changes in the relative numbers of urothelial cells and macrophages are also investigated. Keratin 17 is used as an urothelial protein marker and F4/80 as a macrophage marker because it is the only well studied macrophage marker that does not overlap in specificity with fibroblasts [9]. Moreover, fibroblast-specific protein 1 (FSP1) is used to detect fibroblasts because it is specific for fibroblasts [10] whereas vimentin is also expressed in myofibroblasts and fetal type SMCs [11]. Finally, αSMA is examined through the use of immunohistochemistry rather than transgenic mice. A summary of these markers is presented in Figure 6 and differences between this research and the presented research are further detailed below.

**Modifications in Materials and Methods**

*Animal care.* Aged wild type female mice are acquired from the National Institute on Aging (NIA). In total, 30 mice are ordered, such that on the date of the experiment there will be approximately 10 each of 2 month, 12 month, and 24 month old mice.

*Tissue collection and dissociation.* Mice are euthanized with CO₂. After saturating the abdomen with 70% ethanol, the bladders are exposed, taking care to avoid puncturing the liver while also excising fat and connective tissue. The bladder is held in place with forceps while a 30G1/2 needle is used to inject 250 µL dissociation solution (collagenase 2 in PBS, 2 mg/mL final concentration) into the bladder lumen. Throughout the next 5 min, additional injections of dissociation solution are made in different regions of the bladder between the urothelium and
smooth muscle layer. This should require approximately 3 mL dissociation solution. Exactly 5 min after the first injection, the bladder is extracted, placed in a 60x15 mm culture dish, and incubated at 37°C for 10 min. It is then minced with scissors in the hood and again incubated at 37°C for 5 min. The tissue sample is then gently and slowly titurated with a 1 mL pipette 10 times and again incubated at 37°C for 5 min. The tituration is repeated and the resulting cloudy solution is filtered through a 0.2 µm cell strainer into a 50 mL falcon tube. Cold PBS is used to wash the culture dish until the 50 mL falcon tube contains a total volume of 10 mL. This suspension is poured into a 15 mL falcon tube and 5 mL of cold PBS is used to wash the 50 mL falcon tube. The 15 mL falcon tube is centrifuged at 1400 RPM for 5 min. 13 mL of the supernatant are then extracted and the primary cell pellet is resuspended using a 1 mL pipette. 15 mL of cold PBS are added to the resuspended pellet and the suspension is centrifuged as before. 14.5 mL of the supernatant are extracted and the cell pellet is again resuspended using a pipette. Cell count is then ascertained using 20 µL of this suspension and Trypan blue under 10x magnification. A minimum cell density of $10^5$ cells/mL will achieve best results.

**Immunohistochemistry.** Antibodies against α-SMA (Abcam – ab8211), FSP-1 (Abcam – 2795), keratin 17 (Cell Signaling – 4543), and F4/80 (ebioscience – 47-4801) are used and conjugated to FITC (preconjugated), Alexa Fluor 680 (Invitrogen – A20172), Pacific Blue (Invitrogen – P30013), and APC-Cy7 (preconjugated) respectively (Figure 6). As illustrated in Figure 7, these fluorophores do not significantly overlap. Prior to staining the dissociated bladder with these antibodies, titrations of each antibody in a cell line of each investigated type of tissue will be conducted to determine the ideal antibody concentrations.
Table 6. Summary of Markers and Fluorophores Used to Analyze the Effects of Aging on Bladder Cellular Composition. 4 tissue types are examined.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antibody</th>
<th>Fluorophore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth Muscle</td>
<td>αSMA</td>
<td>FITC</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>FSP-1</td>
<td>Alexa Fluor 680</td>
</tr>
<tr>
<td>Urothelium</td>
<td>Keratin 17</td>
<td>Pacific Blue</td>
</tr>
<tr>
<td>Macrophage</td>
<td>F4/80</td>
<td>APC-Cy7</td>
</tr>
</tbody>
</table>

**Sample preparation for FACS analysis & tissue characterization.** Sample preparation for FACS analysis and tissue characterization will be performed as done earlier, after first establishing compensation controls for each of the antibodies used.

**FUTURE DIRECTIONS**

To strengthen these findings, the presented experiments should be repeated using larger sample sizes. Moreover, bladders were examined 1 week post surgery and the long term impact of bilateral ovariectomy and pBOO should also be investigated.

The changes in cellular composition accompanying bladder injury can be better characterized by using additional marker proteins to target different cell types as well as different levels of differentiation within a single cell type. However, in addition to financial considerations, there are several limitations to the number of antibodies that can be used simultaneously. First, only 3 intracellular proteins can be stained simultaneously without obtaining interference. Furthermore, there are limits to the number of non-overlapping fluorophores available for conjugation. Similarly, not all antibodies of interest are commercially available. For example, although smoothelin is an ideal marker for a mature contractile SMC...
phenotype [5] and would therefore be a very valuable marker for tissue characterization, it is not commercially available.

Studies should also be performed to better characterize the endogenous fluorescence exhibited in transgenic mice. Endogenous fluorescence from transgenic mice may be indicative of whether or not a particular protein is being synthesized, that is if its promoter is activated, rather than whether or not the protein is present in the cell as is ascertained by immunohistochemistry. The efficacy of the endogenous fluorescence as a marker can be determined by treating tissue samples from transgenic mice with conjugated antibodies for the marker of interest and then comparing the fluorescence from both. If the endogenous fluorescence proves to be an adequate marker then more antibodies can be used. Plans to complete an experiment of this nature are already forming, which is why mcherry, the fluorophore for αSMA in the transgenic mouse model, is included in the fluorescence spectrum (Figure 7) with the other fluorophores being used in the current aging study. If the endogenous fluorescence is confirmed as an adequate marker for αSMA, then the fluorophore FITC could be conjugated to another marker, such as calponin, rather than αSMA.
Finally, the tissue characterization techniques established throughout these experiments can be used to analyze changes in the bladder cellular composition associated with a variety of treatments such as current plans to demonstrate how MIF tautomerase inhibitor (ISO-1) partially relieves bladder muscle loss and fibrosis following bilateral ovariectomy. Macrophage migration inhibitory factor (MIF) is a cytokine that is released by the urothelium into the bladder lumen following injury and is an essential component of the inflammation processes [12]. ISO-1 blocks MIF by blocking its tautomerase activity. The aim of the presented research is to better characterize DU pathophysiology and pathogenesis and further studies of ISO-1 and MIF could therefore be very valuable because MIF is essential to the pathways responsible for the

![Fluorophores Used to Analyze the Effects of Aging on Bladder Cellular Composition.](www.bdbiosciences.com)

This figure was generated using the fluorescence spectrum viewer program available at www.bdbiosciences.com and illustrates that the fluorophores being used in the aging study do not significantly overlap. Pacific Blue is conjugated to anti-keratin 17, FITC to anti-αSMA, Alexa Fluor 680 to an antibody against FSP-1, and APC-Cy7 to an antibody against F4/80. Although wild type mice are used, cherry is endogenous fluorescence of αSMA and included above for future studies with transgenic mice.
characteristic attributes of DU that result from bladder muscle stretch, lack of estrogen, and aging.

CONCLUSION

1 week post ovariectomy, smooth muscle degeneration and fibrosis of the bladder are evident. The smooth muscle degeneration resembles a dedifferentiation in which SMC phenotypes transition towards less mature SMC phenotypes. Further studies are needed to better characterize both the endogenous fluorescence in transgenic mice and the changes in cellular composition of the bladder in DU pathogenesis and pathobiology.

ACKNOWLEDGEMENTS

I would like to thank Dr. George Kuchel for his support and guidance throughout this project as well as for enthusiastically inviting me to join his research team despite my limited experience. I would also like to thank Qing Zhu and Xin Zhou for their assistance throughout the entire project. Additionally, I am very grateful for the oversight, patience, and wonderful ability to provide comprehensive explanations that Anthony DeAngelis has shared throughout the current aging study. Finally, this project would not have been possible without Ivo Kalajzic and David W. Rowe, who provided the double transgenic mice.
REFERENCES


