Dissecting the Role of Epithelial vs. Stromal Estrogen Receptor α in Lower Urinary Tract Dysfunction

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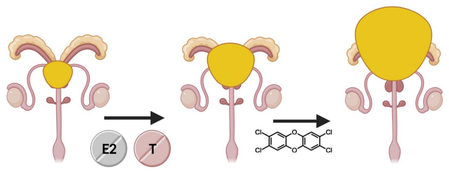
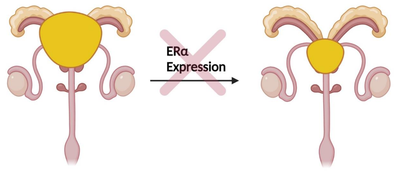
Background

Aging men experience Benign Prostatic Hyperplasia associated with Lower Urinary Tract Symptoms (BPH/LUTS) as a result of pathological changes in the prostate which are attributed to an age-related increase in the ratio of estradiol to testosterone has been shown to be associated with lower urinary tract dysfunction in mice.

Our group and collaborators have shown that *in utero* and lactation exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) sensitizes mice treated with testosterone (T) and 17b-estradiol (E2) to voiding dysfunction which leads to increased bladder volume.

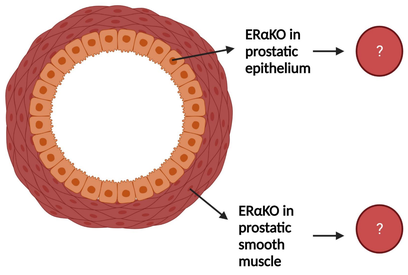


Our group has also shown ERα to be essential in the progression of lower urinary tract dysfunction [3].



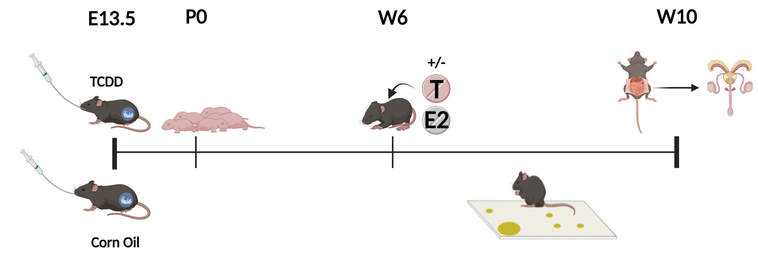
Objective and Hypothesis

ERα has been found in the prostatic epithelium and stroma. Although we have previously established the role of systemic ERα in LUTD progression, we have not determined what cell compartment plays a role in disease progression.



Objective: The objective of this study was to determine the respective roles of epithelial and stromal ER-α in the progression of LUTD induced by testosterone and estradiol or their combination with *in utero* TCDD exposure in male mice.

Materials and Methods



ERα$^{lox/lox}$ mice (B6(Cg)-*Esr1*$^{tm4.1Ksk}$) were bred with transgenic mice expressing the Cre enzyme under the control of the Sonic hedgehog (B6.Cg-*Shh* $^{tm1(EGFP/cre)Cjt}$/J) or the Transgelin promoter (B6.Cg-Tg(*Tgln*-cre)1Her/J) to generate mice with ERα deletion in Shh-expressing cells (*Shh*-Cre/ERα$^{lox/lox}$), or Sm22-expressing cells (*Tgln*-Cre/ERα$^{lox/lox}$). Dams were dosed with TCDD (1 ug/kg) or Corn Oil (CO, 5 mL/kg) when their pups were E13.5. Male pups were aged to six weeks and underwent a testosterone and 17b-estradiol hormone pellet implantation surgery or a sham surgery. Void Spot Analysis was done for four weeks. At ten weeks of age mice were euthanized and lower urinary tracts were collected.

Results

Neither Epithelial Nor Stromal Smooth Muscle Loss of ERα Significantly Affects Void Spot Count in Response to Steroid Hormone Treatment

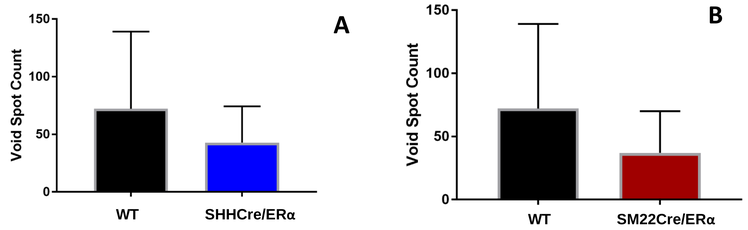


Fig 1. In CO/T+E2 treated mice, neither epithelial nor stromal deletion of ERα resulted in a void spot difference. Void spot count did not significantly differ with epithelial (**A**) or stromal smooth muscle (**B**) loss of ERα in CO/T+E2 mice. Statistical significance was determined by a Student's t test with p<0.05 accepted as significant.

Epithelial Loss of ERα Results in a Significant Reduction in Void Spot Count in Response to Dioxin and Steroid Hormone Treatment

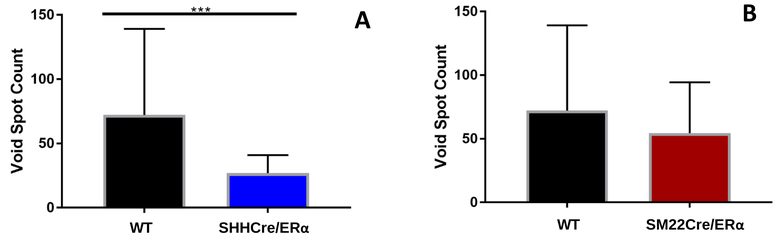


Fig 2. Epithelial deletion of ER-α resulted in a significant void spot reduction in TCDD/T+E2 treated mice. Epithelial loss (**A**) of ERα resulted in a significant reduction in void spot count, while there was no significant difference in the deletion of ERα in stromal smooth muscle (**B**) in TCDD/T+E2 mice. Statistical significance was determined by a Student's t test with p<0.05 accepted as significance. ***: p<0.001

Neither Epithelial Nor Stromal Smooth Muscle Loss of ERα Significantly Affects Bladder Mass Change in Response to Steroid Hormone Treatment

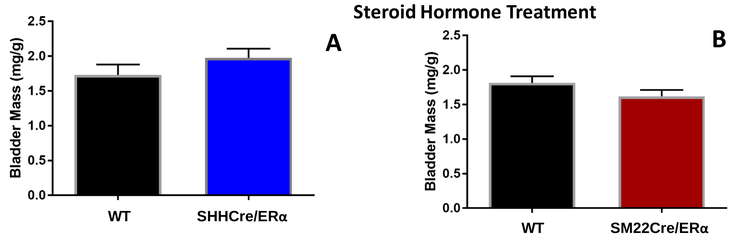


Fig 3. Bladder masses did not significantly change with either the epithelial or stromal deletion of ER-α in CO/T+E2 treated mice. Bladder mass did not significantly differ with epithelial (**A**) or stromal smooth muscle (**B**) loss of ERα in CO/T+E2 mice. Statistical significance was determined by a Student's t test with accepted p<0.05 as significance.

Results (cont.)

Stromal Smooth Muscle Loss of ERα Results in a Decrease in Bladder Mass in Response to Dioxin and Steroid Hormone Treatment

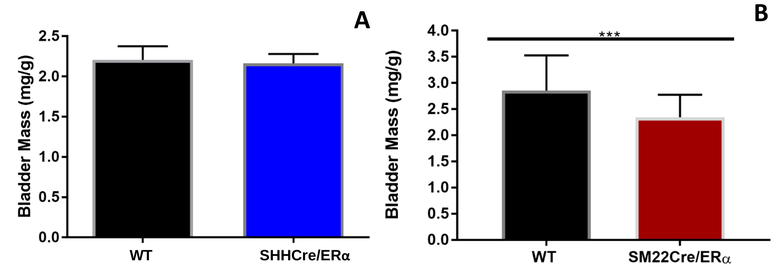
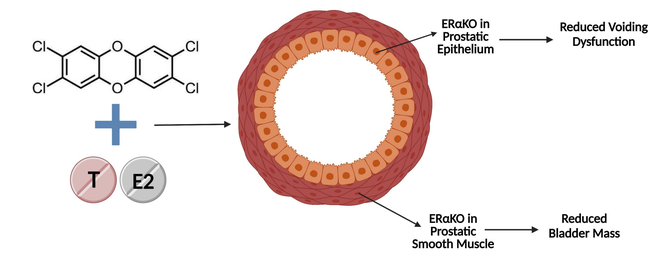


Fig 4. A stromal smooth muscle deletion of ERα resulted in a bladder mass decreased in response to TCDD/T+E2 treatment. . Epithelial loss (**A**) of ERα did not result in a significant bladder mass change, while stromal smooth muscle loss (**B**) resulted in a decreased bladder mass in TCDD/T+E2 mice. Statistical significance was determined by a Student's t test with p<0.05 accepted as significance. ***: p<0.001

Discussion



Conclusion:

- Neither stromal nor epithelial ERα deletion alleviates LUTD in CO/T+E2 mice
- ERα deletion in multiple cell types may be necessary to reduce dysfunction in CO/T+E2 mice
- Smooth muscle and Epithelial ERα may have different roles in the double hit model
- TCDD/T+E2 treated mice show reduced voiding dysfunction with an epithelial deletion of ERα and decreased bladder mass with a smooth muscle ERα deletion.
- Future directions will include looking at total collagen, proliferation, and smooth muscle thickness

References

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2. Turco AE, Thomas S *et.al.* In utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure exacerbates urinary dysfunction in hormone-treated C57BL/6J mice through a non-malignant mechanism involving proteomic changes in the prostate that differ from those elicited by testosterone and estradiol. Am J Clin Exp Urol. 2020 Feb 25;8(1):59-72.
3. Nicholson TM *et.al.* Estrogen receptor-α is a key mediator and therapeutic target for bladder complications of benign prostatic hyperplasia. J Urol. 2015 Feb;193(2):722-9.
4. BioRender.com
5. Graphpad Prism was used to generate results

Acknowledgements

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