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OVEREXPRESSION OF ESTROGEN RECEPTOR B IN BLADDER UROTHELIUM PROTECTS AGAINST UROPATHOGENIC E. COLI URINARY TRACT INFECTION

Hypothesis / aims of study

One in two women experience urinary tract infection (UTI) at least once in their lifetime, and after menopause one in four women with UTI develop recurrence. Treatment for UTI primarily centers on antibiotics which target uropathogens. Of the approaches that leverage host defense factors, topical estrogen administered transvaginally has been shown to reduce UTI in randomized controlled trials. To hone in on estrogen's effect on the bladder urothelium [1] *in vivo*, we created a mouse model with overexpression (OE) of estrogen receptor β (ERβ) restricted to the urothelium (uERβ-OE^{+}). We hypothesize that uERβ overexpression facilitates recovery form UTI, and we aim to ascertain whether female uERβ-OE^{+} mice demonstrate faster bacterial clearance when challenged with uropathogenic *E. coli* (UPEC).

Study design, materials and methods

A transgenic uERβ-OE^{+} mouse line was created by linking the urothelium-specific uroplakin II (UPII) promoter to the ERβ gene (Figure 1) utilizing a C57BL/6 genetic background. Female 4-6 month-old uERβ-OE^{+} mice were compared to female age-matched wildtype (WT) and negative, non-transgene carrying (uERβ-OE^{neg}) littermates in this study. Urinary tract infection was simulated using transurethral inoculation of UPEC (clinical isolate UTI89) at a dose of 1 x 10^{8} colony forming units (CFU) in 50 µL of phosphate buffered saline (PBS) administered intravesically under anesthesia using established protocol [2]. Urine specimens were collected 1, 2, 3, and 4 days post UPEC inoculation and plated on agar in serial dilution to determine bacterial load. After the last urine collection on Day 4, mice were euthanized, and their bladders and kidneys were harvested, homogenized, and plated for bacterial count.

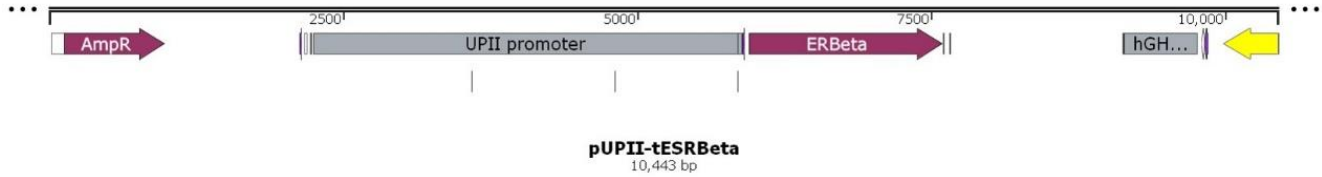

Figure 1. Plasmid sequence of uERβ-OE^{+} with Uroplakin Promoter II (UPII) upstream.

Results

Bladder urothelium was isolated from bladder mucosa using our unique dissection technique [3], and urothelial expression of ERβ (Figure 2A) was shown to be significantly increased in uERβ-OE^{+} mice on reverse transcription of urothelial ribonucleic acid (RNA) and quantitative polymerase chain reaction (qPCR) analysis. In addition, overexpression of ERβ was shown to be specific to the urothelium, given the absence of differential ERβ expression in other bladder tissues or other organs (Figure 2B) from uERβ-OE^{+} mice as compared to those from wildtype. Histologic examination showed no significant differences in bladder urothelium or stroma among the three cohorts.

Female uERβ-OE^{+} mice clear urinary UPEC load (Figures 3A, B) significantly more quickly than uERβ-OE^{neg} and WT controls. A significantly lower bacterial count was also measured in bladders and kidneys (Figures 3C, D) harvested from uERβ-OE^{+} mice on Day 4.

Interpretation of results

Increased urothelial ERβ expression confers significant protection against UPEC.

Concluding message

The uERβ-OE^{+} murine model serves as a valuable new tool to identify pathways to be harnessed for future UTI treatment paradigm, without the direct use of antibiotics or estrogen.

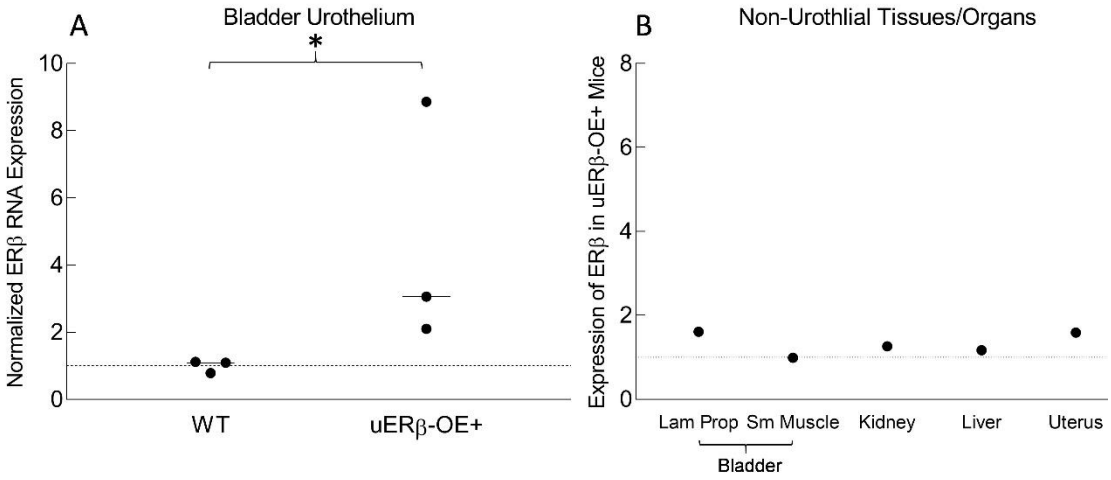

Figure 2. Overexpression of Estrogen Receptor β (ERβ) in uERβ-OE⁺ mice specific to the urothelium. **A**. RNA expression of ERβ in bladder urothelium of uERβ-OE⁺ mice (n = 3) normalized to that of WT (n = 3). Bars indicate median expression, and asterisk indicates statistical significance ($p \leq 0.05$) on one-tailed Mann-Whitney test. **B**. RNA expression of ERβ in other bladder tissues or organs from uERβ-OE⁺ mice normalized to that of WT: lamina propria (Lam Prop) and smooth muscle (Sm Muscle) of bladder and kidney, liver, and uterus. Dotted line denotes WT expression level at 1-fold.

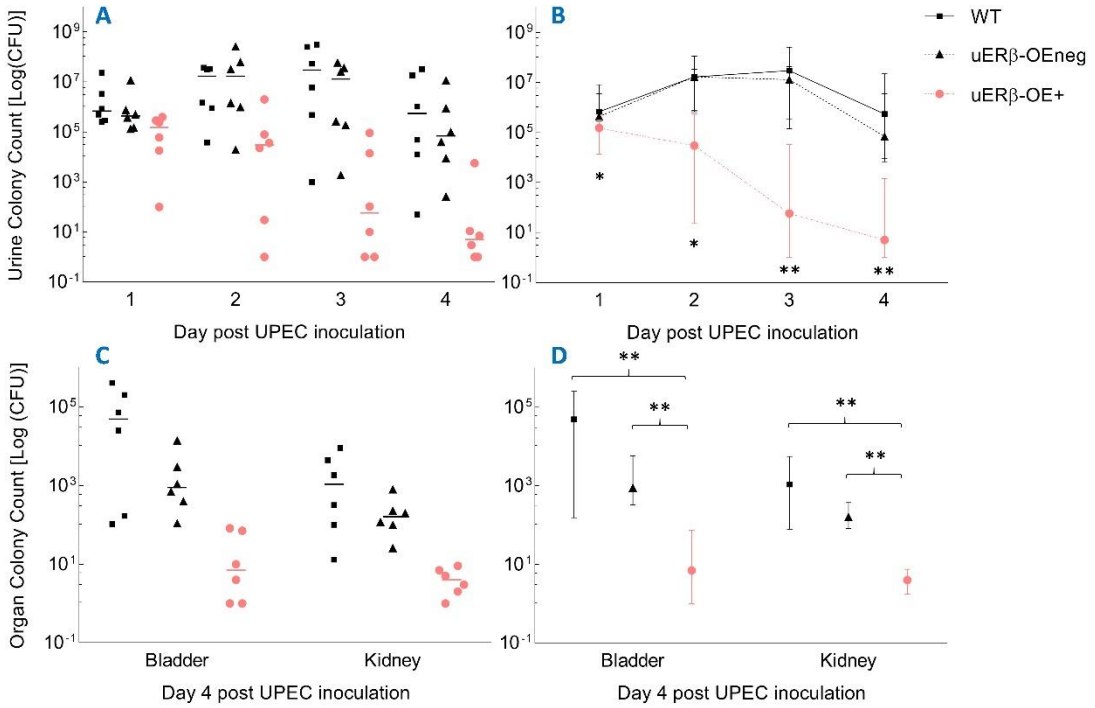

Figure 3. Colony count of urine specimens and harvested organs post Uropathogenic *E. coli* (UPEC) inoculation. Scatter plot with median colony forming units (CFU) of daily urine specimens (A) and homogenized bladders and kidneys (C) harvested on Day 4 from mice (n = 6) in each cohort; summary plot of urine (B) and organ (D) CFU with error bars showing median with interquartile range (IQR) and asterisks indicating statistical significance (* for $p < 0.05$, ** for $p < 0.01$) on one-tailed Mann-Whitney test.

References
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2. Hung CS, Dodson KW, Hultgren SJ. A murine model of urinary tract infection. Nat Protoc 2009;4(8):1230-43.
3. Lu M, Chai TC. A method to study bladder urothelial cellular function in situ. Bladder 2014;1(1):e3.

Disclosures
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