

LOSS OF OSTEOPONTIN FUNCTION PREVENTS THE DEVELOPMENT OF URINARY DYSFUNCTION IN *E. COLI*-INDUCED PROSTATIC INFLAMMATION

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Background

Lower urinary tract dysfunction (LUTD) in men and associated symptoms (LUTS) including weak stream, nocturia, incomplete emptying and intermittent or hesitant urination, can adversely affect the patient's quality of life. The progression of LUTS have been linked to age-related changes including alterations in steroid levels, but there is a growing body of evidence demonstrating that chronic inflammation has a central role in its pathogenesis, in part, by stimulating prostatic fibrosis. Fibrotic pathology is associated with the accumulation of collagen fibers in the stroma and the rearrangement of the periurethral prostatic architecture often resulting in obstructive urinary symptoms. Current therapies do not target fibrosis, however, it is a key event in driving medical resistance to LUTS. Consequently, the development of novel medical strategies requires a better understanding of the molecular aspects of this prostatic disease.

Our previous study identified osteopontin (OPN) as a novel gene in the pathogenesis of benign prostatic diseases. We identified, that osteopontin secretion is induced by inflammatory cytokines and it stimulates multiple cytokines and chemokines attracting immune cells and exacerbating inflammatory responses. In the current study we investigated whether prostatic fibrosis and urinary dysfunction induced by transurethral bacterial instillation is attenuated in the absence of osteopontin.

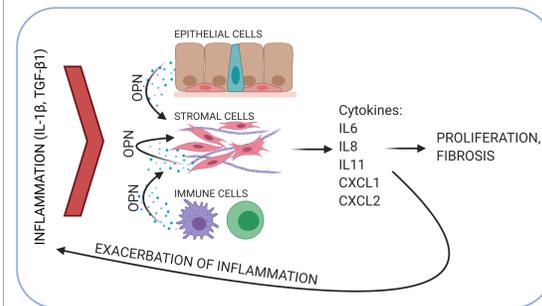


Figure 1.: Proposed action of Osteopontin in the prostate. Osteopontin is secreted from prostate cells in response to inflammatory stimuli and exacerbates inflammatory reactions by triggering cytokines and chemokines from stromal cells leading to fibrosis.

Materials and Methods

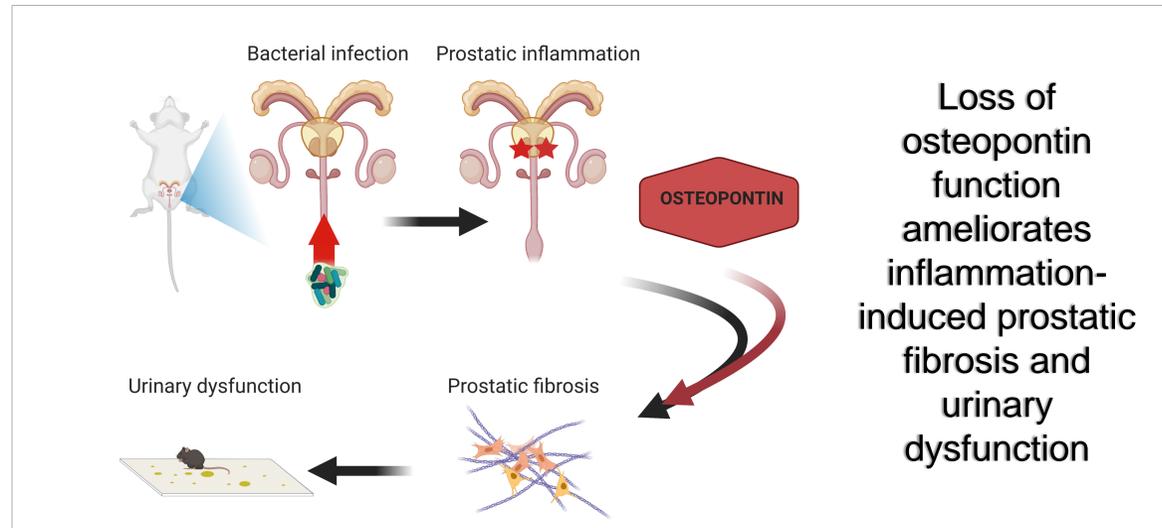
Transurethral instillation of uropathogenic *E. coli*: UT189 *E. coli* strain isolated from a human patient with cystitis was instilled with 100 ul culture with OD 0.8 from an overnight shaking culture. Control mice received PBS. The instillation was repeated 3 days later and urine was sampled next day. We determined that the susceptibility of WT and OPN-KO mice for bacterial colonization is similar by plating urine on agar plates.

Void spot assay: mice were isolated in cages with filter paper for 4 hours weekly. Filter papers were imaged with UV epi-illumination and analyzed with Void Whizzard ImageJ plugin.

Measurement of collagen abundance: tissues were stained with picrosirius red and birefringence was measured using circularly polarized light. The number of green, red, orange and red pixels were calculated in ImageJ and added together to determine the proportion of total collagen/tissue area.



Hypothesis



Results

LOSS OF OSTEOPONTIN SUPPRESSES FIBROSIS IN MOUSE PROSTATE LOBES

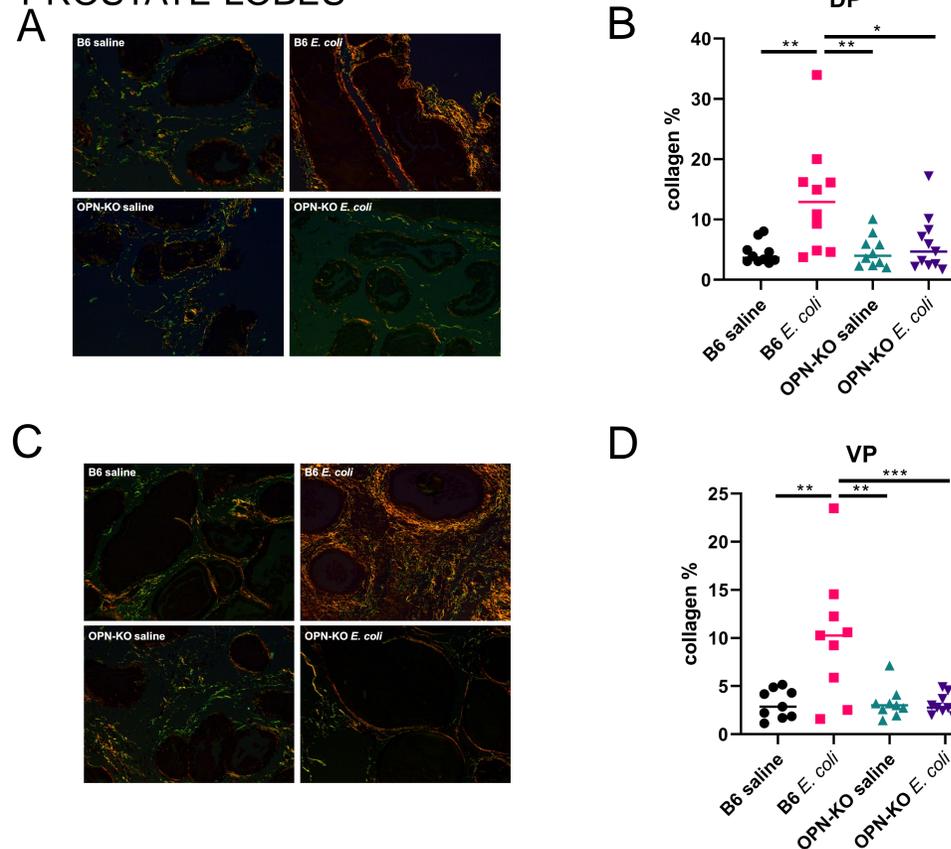


Figure 2.: Inflammation-induced collagen accumulation is significantly reduced in osteopontin knockout (OPN-KO) mice. Collagen abundance was increased in both dorsal (DP, **A** and **B**) and ventral (VP, **C** and **D**) prostate lobes in response to *E. coli* instillation, but significantly reduced in OPN-KO mice. Mice were transurethrally instilled with *E. coli* 2 times, 3 days apart and euthanized 60 days after the first instillation. Prostate lobes were stained with picrosirius red and photographed under circularly polarized light. Collagen area was calculated with ImageJ and normalized to tissue area captured with bright field imaging. Values were averaged for 3 images/sample. Significance was calculated with one-way ANOVA followed by Tukey's post hoc analysis. *p<0.05, ** p<0.01, ***p<0.001

Results (cont.)

LOSS OF OSTEOPONTIN REDUCES *E. COLI*-INDUCED URINARY DYSFUNCTION

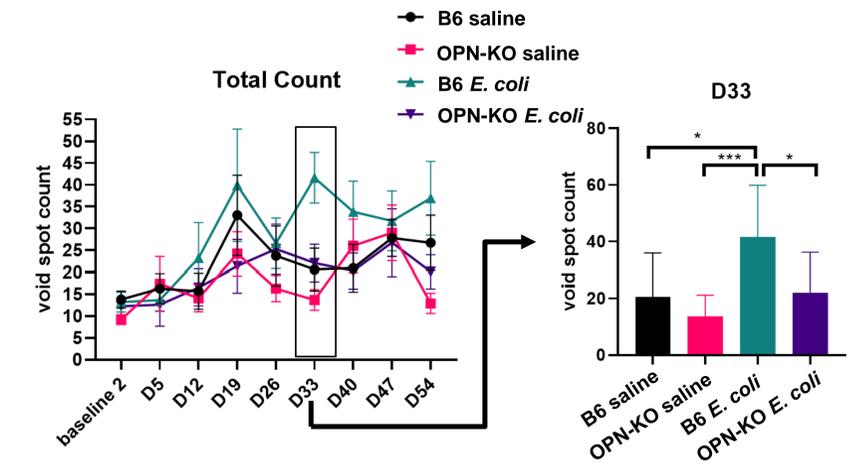


Figure 3.: *E. coli*-induced urinary dysfunction does not develop in osteopontin knockout (OPN-KO) mice. Urinary function was measured using void spot assay in which an increase in void spot frequency indicates dysfunction. During weekly measurements, we found a significant increase in frequency in wild type (B6) *E. coli* instilled mice at day 33, but not in OPN-KO mice. Significance was calculated with one-way ANOVA followed by Tukey's post hoc analysis. *p<0.05, ***p<0.001.

Conclusions & Future Directions

Osteopontin is a key driver of inflammation-induced prostatic collagen accumulation and related urinary dysfunction

Future goals:

- Our preliminary experiments showed that OPN-KO mice reach the same level of bacterial colonization as wild type mice. We aim to decipher whether the development of acute inflammation and fibrosis are also affected in OPN-KO mice or whether the improvement in OPN-KO mice is related to faster resolution of fibrosis.
- We will study periurethral collagen accumulation in wild type and OPN-KO mice which can be directly linked to urinary dysfunction
- We will identify the key molecular pathways affected in OPN-KO mice with RNAseq
- We will test osteopontin-targeting therapies in *E. coli*-induced and other prostatic inflammation mouse models

Acknowledgements

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References

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