Loss of Osteopontin function attenuates immune cell infiltration and collagen accumulation in a steroid hormone-induced lower urinary tract dysfunction model

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Background
- Lower urinary tract symptoms (LUTS) deteriorate the quality of life of a significant proportion of the male population, particularly those >50 years of age.
- Current therapeutics target smooth muscle dysfunction and prostatic enlargement, but resistance may develop. Prostatic inflammation has also been linked to LUTS, but its molecular process is not well understood.
- The age-related increase in estradiol:testosterone ratio leading to hormonal imbalance is likely a key factor in prostatic disease by triggering inflammation in the prostate, but this has not been shown previously.1
- Osteopontin (OPN) is a pro-inflammatory cytokine which we found to be associated with LUTS and demonstrated its role in aggravating prostatic inflammation and fibrosis in mice.2,3
- We hypothesize that osteopontin facilitates the infiltration of immune cells and the development of fibrosis in the prostate triggered by steroid hormone imbalance. We believe these processes contribute to prostatic disease and the development of LUTS.

Materials and Methods
- Male C57Bl/6j (WT) and Spp1−/− (OPN-KO) mice were surgically implanted with subcutaneous T+E2 pellets composed of 25 mg testosterone (T) and 2.5 mg estradiol (E2). Tissues were collected 2, 6, or 12 weeks later.
- Immunohistochemistry (IHC) was performed on ventral prostate (VP) and dorsal prostate (DP) tissues. Type 1 collagen was identified with a Coll1a1 antibody (1:500). Immune cells were identified with common immune cell marker CD45 (1:2000). Images of VP and DP tissue (6/tissue) were taken at 40x magnification. CD45-positive cells were counted manually and was expressed as cells/mm². Tissue from prostates with incidental high-grade inflammation (>400 cells/mm²) were excluded from the dataset for analysis. Significance was determined with one-way ANOVA. **: p<0.01 ***: p<0.001.
- Multiplex fluorescent hybridization chain reaction (HCR) was performed to target genes for macrophages/monocytes (Cd163, Cd19, and Cd3e.), and T-cells (Cd3e).
- Osteopontin deficiency leads to lower prostatic levels of collagen I in chronic steroid hormone imbalance.

Results

Loss of OPN function results in a hindered inflammatory response induced by steroid hormone imbalance

Loss of OPN function results in lower prostatic levels of collagen I in chronic steroid hormone imbalance

Figure 1. Immune cell number significantly increases in response to early (2 week) T+E2 treatment in WT but not in OPN-KO mice. Panel A: Representative images of VP tissue from mice after 2 weeks of T+E2 treatment. Panels B, C, and D: CD45-positive cell counts from the VP and DP after 2, 6, or 12 weeks of T+E2 treatment, respectively. Immunohistochemistry was performed on VP and DP tissues with CD45 to identify immune cells. Images of VP and DP tissue (6/tissue) were taken at 40x magnification. CD45-positive cells were counted manually and was expressed as CD45 cells/mm². Tissues from prostates with incidental high-grade inflammation (>400 cells/mm²) were excluded from the dataset for analysis. Significance was determined with one-way ANOVA. **: p<0.01 ***: p<0.001.

Figure 2. Collagen density significantly increases in response to long-term (12 week) T+E2 treatment in WT but not in OPN-KO mice. Panel A: Representative images of VP tissue from mice after 2 weeks of T+E2 treatment. Panels B, C, and D: Data from Coll1a1 optical density (OD) quantification in the stroma of VP tissues after 2, 6, or 12 weeks of T+E2 treatment, respectively. Immunohistochemistry was performed on VP and DP tissues with Coll1a1 antibody to identify type 1 collagen. Images of VP and DP tissue (3/tissue) were taken at 20x magnification. Coll1a1 OD was calculated via tissue segmentation to differentiate stroma from epithelium. Significance was tested via pair-wise comparison with Mann-Whitney test. *: p<0.05.

Results (cont.)

Loss of OPN function prevents macrophage/monocyte increase in steroid hormone imbalance

Discussion
- Loss of osteopontin function in our steroid hormone imbalance model results in:
  - a hindered inflammatory response
  - lower levels of type 1 collagen, indicative of less fibrotic development
  - decreased presence of macrophages/monocytes
  - unaffected presence of T-cells
- Note: B-cells were not detected in the prostate in our model
- OPN plays a role in aggravating inflammation and fibrosis associated with steroid hormone imbalance.
- Future research: Extend HCR protocol to further understand presence of immune cell subsets.

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References