

Hematuria Regulates Urothelial Response to UTI

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Introduction and Objective: Urinary tract infections (UTIs) is the most common urogenital disease. Hematuria is a common marker of UTI and is thought to be pathogenic because it can deliver heme-iron to bacteria, inducing their growth. The Barasch Lab previously identified a small siderophore chelator, Lipocalin-2 (Lcn2/NGAL), that binds to enterochelin-Fe and prevents bacterial sequestration of molecular iron, but Lcn2 does not bind heme iron. Our research explores the activity of heme-iron in UTI.

Methods: To simulate UTI, mice were inoculated with 10⁷ GFP-tagged UPEC. Thio-uracil labeling of nascent RNA, and RNAScope and immunofluorescence techniques were used to identify iron and heme regulated genes. In addition, we used a Hmox1 reporter mouse and Nile Red-Palladium based probes to detect carbon monoxide (CO).

Results: Superficial urothelial cells demonstrated Lcn2 expression 4-12 hours after infection; concurrently, urine heme was found in large quantities (~500nm) by spectrophotometry, suggesting that Lcn2 expression was induced by urine heme levels. Using nascent RNA capture methods, we identified urothelial Hmox1, RNAScope depicting Hmox1 RNA expression spanning 4-12 hours after infection. Since HMOX1 produces CO gas as a byproduct of heme metabolism, we synthesized a Pd probe and detected bladder emission of CO. Together the data demonstrate that urothelial cells respond to a UTI by sequestering and metabolizing heme and concurrently release siderophore capturing Lcn2. Finally, heme was found to be protective to the urothelium as the superficial cells underwent shedding in the UTI at 12hrs but in the Heme + UTI mice, the urothelium was practically preserved as demonstrated by the CK20 staining (marker for superficial cells) (Figure 1).

Conclusion: Overall, we have identified a pathway by which urothelial cells metabolize heme and release Lcn2 in response to a UTI. Previous studies have shown heme to be a destructive molecule due to heme-iron's catalytic capabilities. However, we find that supplying exogenous heme is highly protective to the urothelial layer. Future studies will be aimed at explaining this phenomenon.

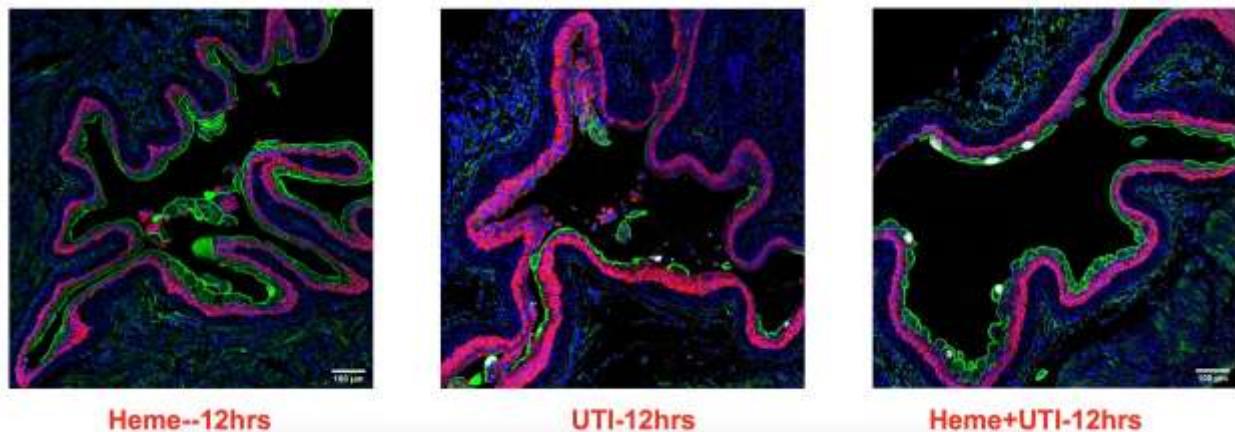


Figure 1: Immunostaining of mouse bladder tissue for Dapi (blue), CK5 (Basal Urothelial Cells, Purple), CK20 (Superficial Urothelial Cells, Green), and bacteria (white).