Developing a Diagnostic Marker for Bladder Outlet Obstruction

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Introduction: Bladder outlet obstructions (BOO) affects an estimated 18.5% of the population and causes urological symptoms such as, weak stream, urge incontinence, and nocturia which can impact one's quality of life [1]. The bladder dysfunction is due, at least in part, to pressure-induced changes in urothelial gene expression. For instance, former lab member Cody Dunton has shown that cyclic elevated pressure caused changes in gene expression indicative of epithelial mesenchymal transition (EMT) and fibrosis [2]. In addition, high pressure voiding of BOO causes blood flow disruption, resulting in tissue ischemia. For this reason, we exposed urothelial cells to transient hypoxic conditions using enzymes or a modular hypoxia chamber and demonstrated that exposure to hypoxia led to an increase in nitric oxide and stabilization of HIF-1α. Although EMT and hypoxia responses of urothelial cells can provide diagnostic markers of BOO, collection of tissue biopsy can be invasive in nature. Recent studies have shown that non-coding RNAs including microRNAs (miRNAs) found in urine offer a potential biomarker for BOO [3]. Siebenthal et al. have identified three miRNAs that are upregulated in urinary exosomes of BOO patients [4]. In this study we plan to develop a method for isolating exosomes from urothelial cell supernatant and compare the exosomes resulting from pressure cycling.

Methods: Hypoxic media was prepared with 0.5 μg/mL GOX in F-12K (VWR) media as well as catalase (CAT, 120 U/mL) and HEPES (25 mM). Rat urothelial cell line (MYP3 cells) were exposed to either enzyme-induced hypoxia (GOX/CAT media) or gas-induced hypoxia (hypoxia chamber: 5% O2, 5% CO2, balanced with nitrogen) for up to 2 hours. For exosome isolation, MYP3 rat cells were seeded in 15cm dishes. Once cells reached ~70% confluency, media was replaced with serum free media, and cells were exposed to pressure regimes characteristic of chronic BOO for 24 hours. Cells were either exposed to no pressure (CTRL) or cyclic pressure consisting of a storage pressure of 15 cmH_2O for 175 min and a voiding pressure of 75 cmH_2O for 5 min (PRES). The supernatant from these experiments were collected and used for exosome isolation following previously established differential centrifugation method [5].

Results and Discussion: Under both enzyme and gas-induced hypoxia, HIF-1α was stabilized and there was an increase in nitric oxide after 1 hour of exposure. MYP3 cells exposed to high pressure cycling resulted in a visible pellet after differential centrifugation; whereas cells exposed to no pressure didn't exhibit a pellet. Furthermore, there was a strong signal for exosome marker CD81 in PRES exosomes. Additional optimization to our experimental design is needed to capture exosomes from the no pressure control. Future work entails extending the serum starvation period to elicit more exosomes from the no pressure control.

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