Understanding the molecular mechanisms driving benign prostatic hyperplasia through spatiotemporal proteomic analysis

George M. O'Brien Urology Research Center
Department of Urology
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

School of Pharmacy
UNIVERSITY OF WISCONSIN–MADISON

Hannah N. Miles[1,2,3], Alexis E. Adrian[2,3,4], Teresa T. Liu[2,3,4], Lingjun Li[1,5], William A. Ricke[1,2,3,4]

[1]School of Pharmacy, [2]Department of Urology, [3]George O'Brien Urology Research Center,
[4]School of Medicine and Public Health, [5]Department of Chemistry, University of Wisconsin-Madison

Li Research Group

Introduction and Objectives

Benign prostatic hyperplasia (BPH) is a disease of the prostate that affects a vast majority of the aging male population. Though BPH is non-malignant in nature, the associated lower urinary tract symptoms (LUTS) that can develop significantly decrease quality of life and burden the healthcare industry. Many of these symptomatic cases are pharmacologically targeted using 5-reductase inhibitors (5ARIs), but these compounds alter the steroid hormone environment and may work against endogenous mechanisms protecting against symptomatic development. Here, we investigated a hormone-induced mouse model of BPH through mass spectrometry imaging (MSI) techniques to further understand the local steroid hormone environment and identify mechanisms protecting or driving disease progression.

Methods

C57BL/6J mice (24 months of age) were surgically implanted with compressed hormone pellets containing 25 mg testosterone (T) and 2.5 mg 17-estradiol (E_2) for 4 weeks. After euthanasia, the urogenital tract was dissected to excise the urethra, prostate lobes and bladder, which were fixed in 10% neutral buffered formalin for 24 hours at room temperature. After dehydration and embedding in paraffin, transverse sections (12 µM) were cut and mounted onto ITO-coated slides. Once mounted, the slides were heated and re-hydrated to remove paraffin before antigen retrieval in 20 mM citric acid buffer. On-tissue digestion and matrix application were performed using a robotic TM sprayer system, then slides were imaged using a MALDI LTQ Orbitrap mass spectrometer from Thermo Scientific.

Results

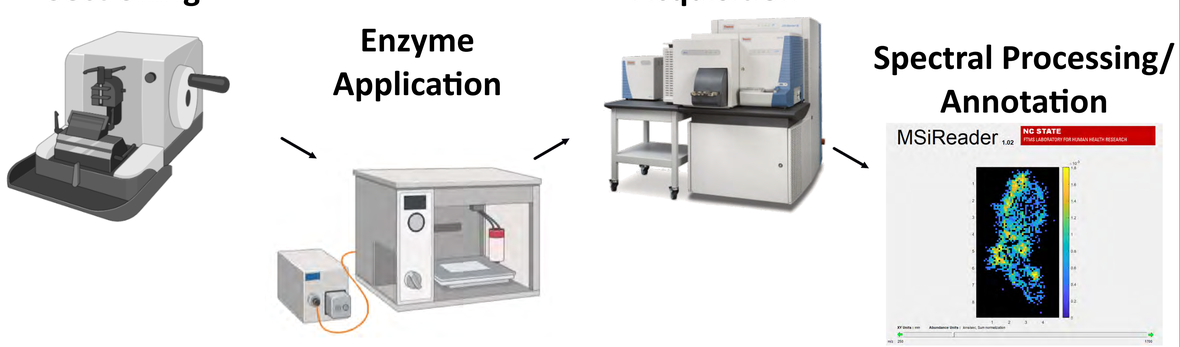

Figure 1. Overview of the workflow from tissue mounting onto ITO-coated slides through the spectral annotation and processing using the open-source MSiReader software.

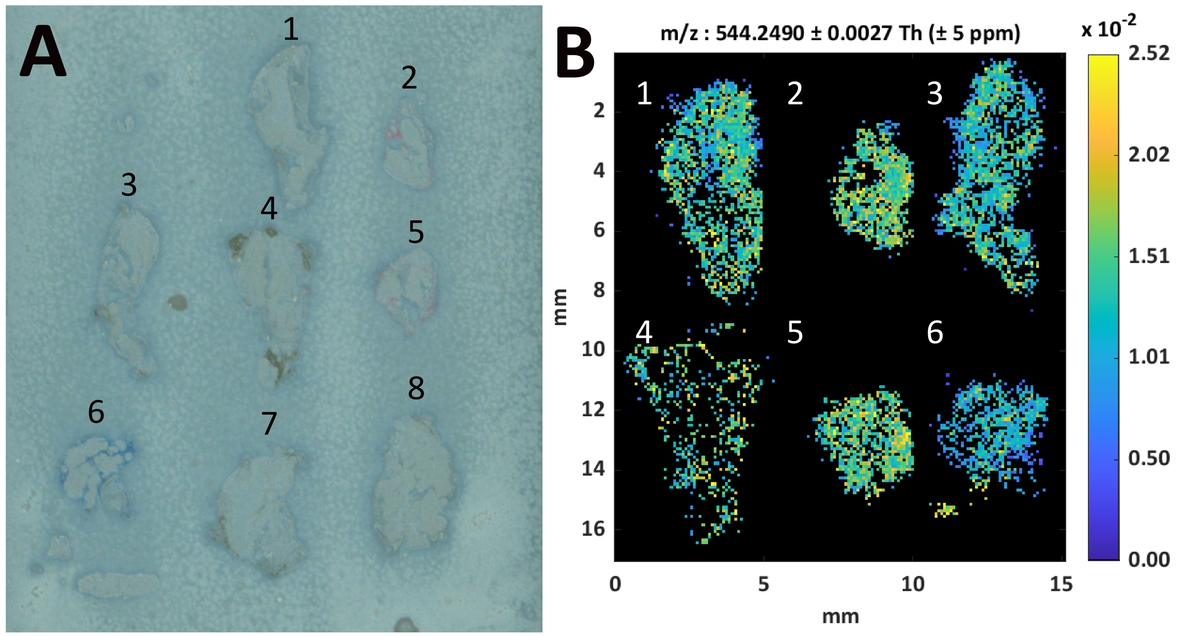

Figure 2. Scanned and spectral images of the anterior prostates of eight 24-month old mice, either untreated (n=2) or treated with T+E_2 (n=6). Due to poor spectral quality and high signal-to-noise, tissues 7 and 8 were omitted from further analysis, leading to n=4 for the T+E_2 treatment group. **A.** Scanned images of the eight anterior prostate sections prior to MALDI-MSI analysis. Tissues were sectioned at a thickness of 12 µm and mounted onto ITO-coated slides prior to on-tissue tryptic digestion and matrix application. **B.** Representative MALDI-MSI images of anterior prostate tissues using the singly-charged Col4a3 peptide of 544.2490 m/z. Matrix used was α-Cyano-4-hydroxycinnamic acid (CHCA). Images were obtained using a mass resolution of 15,000 and a spatial resolution of 100 µm, with each individual pixel indicating a 100 µm spectral section. The image was normalized using total ion current (TIC) values obtained over all spectra for the specified m/z value.

Results (cont.)

Table 1. Anterior prostate tissue numbers along with the corresponding mouse and treatment group information.

Tissue No.	Mouse	Treatment
1	B937	T+E_2
2	B934	Untreated
3	B942	T+E_2
4	B938	T+E_2
5	B935	Untreated
6	B943	T+E_2
7	B939	T+E_2
8	B937	T+E_2

Conclusions and Future Directions

Through this work, localization of key proteomic players in BPH development and progression will be identified that allow further insights into disease mechanisms. Further optimization of sample preparation protocols, instrument methods as well as instruments used for imaging are currently underway to improve spatial and mass resolution of tissue images. Additionally, confirmation of annotated spectra will be done using on-tissue MS/MS or using LC-MS/MS via peptide extraction protocols.

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