Profiling differences in secreted and intracellular proteomic expression in a cellular model of benign prostatic hyperplasia

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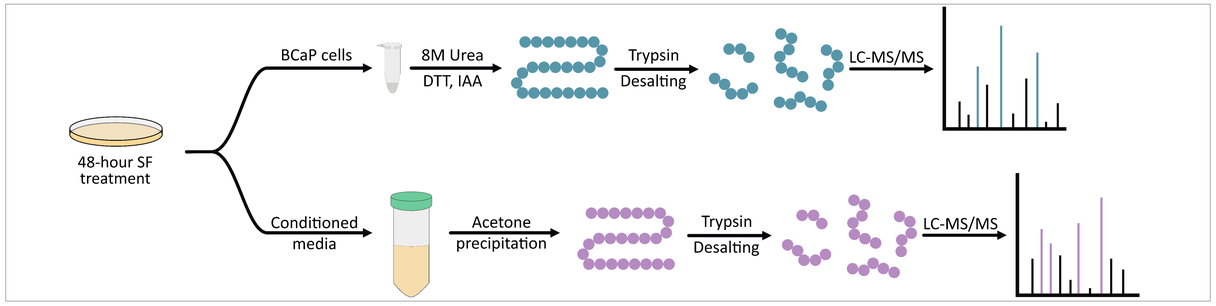
Introduction and Objectives

Benign prostatic hyperplasia (BPH) and prostate cancer are two distinct conditions that influence prostatic proteome composition. While these conditions develop as men age, pathogenesis is markedly different; thus, a deeper understanding of the molecular mechanisms driving progression is crucial. Recently, a BPH1-derived cancer progression (BCaP) model was created to map the various molecular changes that occur across stages of prostate cancer.[1] The use of BPH1 cells as the precursor allows for a clean comparison of genetic and molecular differences between the two disease states, furthering our understanding and treatment of such conditions. Here, we set out to comparatively examine cellular models of prostate protein expression – in the intracellular proteome and the secretome at various stages of prostate disease. While this analysis is exploratory, we expect to see marked differences in expression across all four stages of progression.

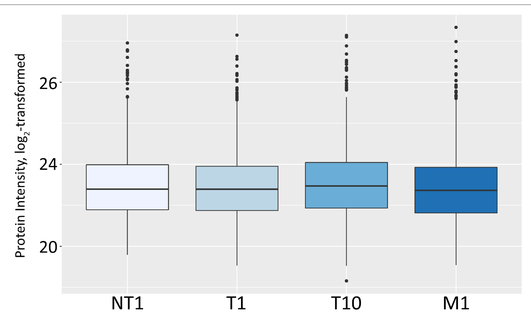
Methods

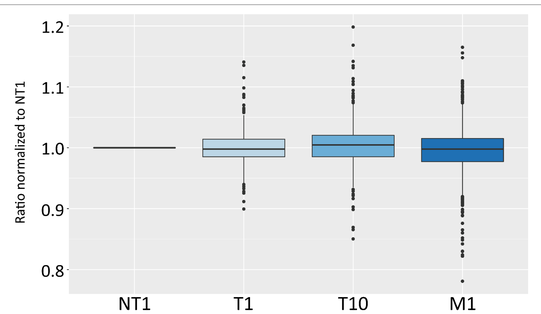
Four BCaP model lines (NT1, T1, T10 and M1) were maintained in DMEM/F-12 medium supplemented with 5% FBS and 1X penicillin/streptomycin. Once sub-confluent, cells were subjected to two 24-hour treatments in serum-free media. At 48 hours, conditioned media and cells were harvested and stored at -80°C until sample preparation. Cell lysis was achieved via sonication using urea buffer and proteins were reduced and alkylated via DTT and IAA, respectively. Secreted proteins were concentrated via acetone precipitation and reduced/alkylated as described above. 500 µg of protein was taken for overnight tryptic digestion and peptides were purified via C18 cartridges. Samples were analyzed in duplicate using LC-MS/MS on a nanoAcquity UPLC coupled to a Q-Exactive quadrupole orbitrap mass spectrometer. Peptides were fragmented using higher-energy collision dissociation (HCD) then identified and quantified using both the PEAKS and MaxQuant software programs. Data analyses were carried out with the use of the MaxQuant software extension Perseus.[2,3]

Methods (cont.)

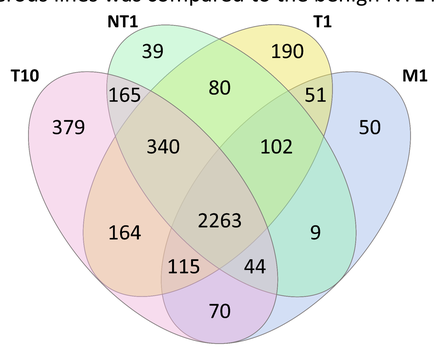


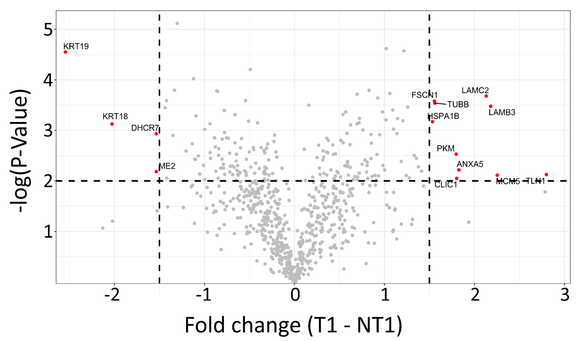
Results

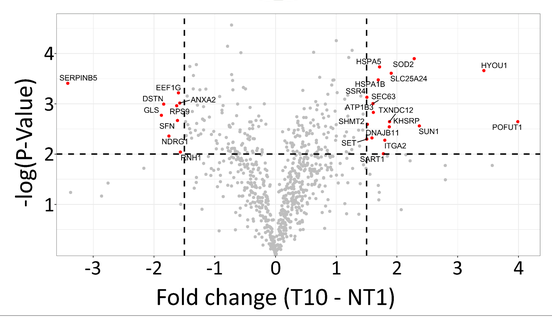


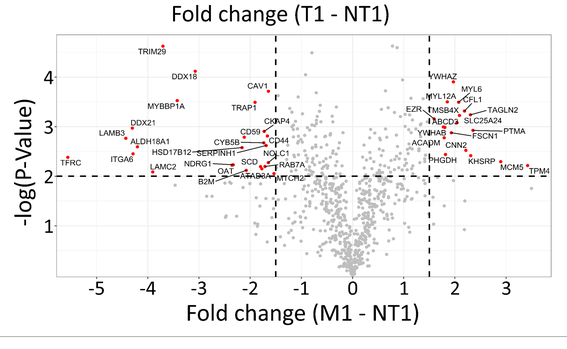


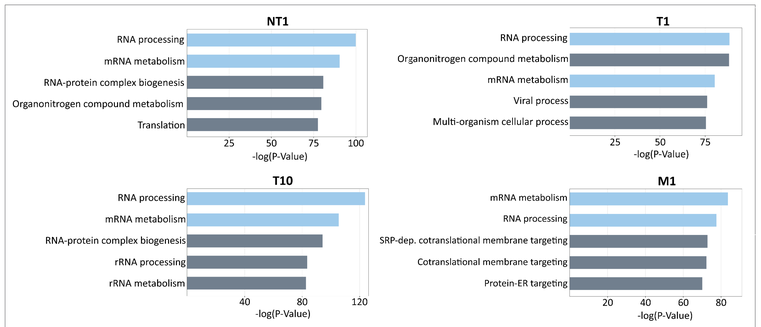
Using the PEAKS software, we identified approximately 4000 proteins across all four samples, with just over 2200 of those identified in all cell lines. Of these identified proteins, around 900 proteins were quantifiable for further analysis. Each of the cancerous lines was compared to the benign NT1 line for identification of significantly up- and downregulated proteins.











Using the identified proteins from each cell line, a gene ontology (GO) enrichment analysis was performed to map quantifiable proteins to their associated biological processes. The top five enriched processes are shown above for each cell line, with many of these enriched processes being shared across lines. Processes such as mRNA metabolism and RNA processing were highly affected across all four lines and are highlighted above for each, but the metastatic M1 cell line demonstrated the highest level of uniquely affected processes in relation to the remaining three lines.

Conclusions and Future Directions

This exploratory analysis allowed insights into the cellular proteomics differentiating cancerous from benign prostate tissue. Such insights will help in the development of new, more targeted therapies for both BPH and prostate cancer. Due to limited time and instrument availability, only the intracellular proteomics were investigated here, but examination of the secretome is ongoing. Future directions involve the inclusion of the BPH-1 cell line to comparatively examine differences in expression with the benign NT1 BCaP line. Once similarities and differences in proteomic signature are established between both intracellular and secreted proteins, more targeted analyses will be pursued to isolate potential therapeutic targets.

Acknowledgements

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References

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