Functional characterization of androgen receptor mediated transcription

Shreyas Lingadahalli[1,*], Tunc Morova[1,*], Flora Huang[1,*], Ivan Pak Yu[1], Dogancan Ozturan[2], Eugene Hu[3], Colin Collins[1], Eldon Embrely[3], **Nathan A. Lack[1,2]**

1) Vancouver Prostate Centre, Vancouver, Canada, 2) Koç University, Istanbul, Turkey, 3) Simon Fraser University, Vancouver, Canada

Background: Androgen receptor (AR) signalling is essential to nearly all prostate cancers. Any alterations to AR-mediated transcription can have a profound effect on prostate carcinogenesis and tumour growth. Yet despite this importance, many critical aspects of AR activity are poorly understood. Specifically, there is a stark contrast between the number of AR binding sites on DNA (tens of thousands) and androgen-driven gene transcription (hundreds). Functional validation of these AR binding sites (ARBS) is required to better understand PCa growth and development.

Methods and Results: To characterize how AR induces transcription, we systematically tested the enhancer activity at every clinical ARBS with a novel massively multi-parallel enhancer assay. Interestingly, only 7% of ARBS were found to be AR-mediated enhancers. Demonstrating the importance of this functional approach, these enhancers could not be readily identified by descriptive techniques including epigenetic ChIPseq or DNA accessibility. Further, we observed that a large number of ARBS were constitutively active enhancers that were not affected by AR binding. In preliminary experiments we show that these constitutive enhancers work in concert with androgen-inducible enhancers. Our results demonstrate that each prostate cancer cell line has altered enhancer usage potentially due to co-operative AR binding. Finally combining these results with clinical whole genome sequencing and chromosomal confirmation capture methodologies we identified and validated specific somatic mutations at enhancer sites that impacted gene expression and cancer growth.

Conclusions: Using multiple functional genomic approaches, this work provides the first "map" of AR enhancers and their target genes. Annotation of these non-coding regulatory elements is critical to guide selection of potential driver mutations. In proof-of-principle experiments we demonstrated that a single nucleotide variant at AR enhancer can affect gene expression and cancer growth.

Conflict of Interest: None to declare

Funding Acknowledgements: TUBITAK 1003, CIHR, Vancouver Prostate Centre