ABSTRACT

INVESTIGATING A MECHANISM FOR P38-MAPK REGULATION OF NOTCH IN PROSTATE EPITHELIAL DIFFERENTIATION

By

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Many pathways misregulated in prostate cancer are also involved in epithelial differentiation. However, specific mechanisms for the cellular and molecular origins of prostate cancer remain elusive. Better understanding of these genes and their specific functions in differentiation may enlighten us as to how their misregulation could drive oncogenesis. My thesis work focused on understanding how p38-MAPK drives prostate epithelial differentiation. My primary hypothesis was that p38-MAPK regulation of Notch3, via Myc, is required for normal prostate epithelial differentiation.

Differentiation in the prostate is a homeostatic process between two cell types in an epithelial bilayer: basal and luminal cells. Each layer has its own progenitor population, but there are also bipotent cells capable of basal-to-luminal differentiation. I utilized primary prostate epithelial cells and induced differentiation in vitro to interrogate signaling pathways. I utilized shRNA, pharmacologic inhibition, and constitutive activation to study the effects of manipulating p38-MAPK, Myc, and Notch signaling during differentiation. I created various dox-inducible shRNA and cDNA overexpressing lentiviral constructs. In the process, I modified and improved a commonly used lentiviral dox-inducible shRNA vector, Tet-pLKO-Puro. In addition to modifying the vector, I also created a streamlined protocol for quick and efficient design and screening of cloned shRNAs.

Using my bevy of molecular tools, I investigated p38-MAPK during differentiation. Inhibition (SB202190, BIRB796) or shRNA knockdown of p38α or p38δ prevented formation of a luminal layer. Additionally, treatment with a γ-secretase inhibitor (RO4929097) or shRNA knockdown of Notch1 or Notch3 greatly impaired differentiation and caused premature luminal

cell death. Knowing that p38-MAPK and Notch were required for differentiation, I next investigated how the pathways may be connected.

Activation of p38-MAPK (via a constitutive MKK6 mutant) increased Notch3 mRNA expression. Upregulation of Notch3 was dependent in part on Myc, as siRNA or inhibition of Myc (10058-F4) diminished the effect by more than half. I further investigated transcriptional regulation of Notch3 by validating two enhancer elements using a combination of ChIP, RNAseq, and Luciferase reporter assays. Additionally, I found that p38-MAPK also regulates Notch3

via increased mRNA stability. Lastly, I investigated upstream (ligand) and downstream (Hes/Hey) Notch signaling during differentiation. I observed differential Notch ligand regulation and divergent regulation of several target genes by Notch1 and Notch3.

My findings reveal a new mechanistic link between p38-MAPK and Notch signaling during epithelial differentiation. Moreover, this work demonstrates novel mechanisms of Notch3 regulation at both the transcriptional and post-transcriptional level by p38-MAPK and Myc. Additional experiments suggest Notch3 may play a unique role in driving differentiation by differentially regulating a subset of Notch target genes. Future work will build on these findings and further increase our understanding of these pathways in the prostate, with the ultimate goal of bringing new insights into tumor biology.