

## **RIOK2 IS A MEDIATOR OF OBESITY ENHANCED PROSTATE CANCER GROWTH**

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**BACKGROUND:** Obesity is a growing global and U.S. health problem. For 2012, an estimated 117,000 cancer cases in the U.S. were deemed preventable by achieving and maintaining a healthy weight, including 11% of all advanced prostate cancers (PC). Obesity is associated with greater risk of high-grade PC, recurrence after therapy, metastases, and PC specific death. We exploited this link to identify actionable targets by performing a shRNA genomic screen in obese and lean mice targeting the entire kinome. Our functional screen identified multiple kinases, which appear to be essential for obesity-driven PC growth including kinases previously implicated in PC and others not previously studied such as Right Open Reading Frame Kinase 2 (RIOK2).

**METHODS:** LAPC-4 cells were inoculated with an shRNA library of ~5000 lentivirus targeting 513 kinases. 5x10<sup>6</sup> cells (~1,000 cells per shRNA) were grafted to chronically obese mice. Tumors were established to ~200 mm<sup>3</sup> and a portion collected for reference. Remaining mice were randomized to continue on ad lib WD or 25% CR diet. Genome-integrated shRNA inserts were amplified using nested barcoded primers and sequenced using Illumina Hi-Seq 2000 and quantified. A virtual screen based on a RIOK2 homology model generated using MODELLER based on two RIOK2 crystal structures. Global gene expression analysis of RNA from scramble control and RIOK2 knockdown with two shRNAs in 22RV1 cells was conducted with Affymetrix U133A Plus Array.

**RESULTS:** RIOK2 expression correlates with Gleason grade in radical prostatectomy tissue and RIOK2 kinase activity is elevated in metastatic vs localized PCs. ENCODE ChIP-seq data shows Androgen Receptor and Myc bind to the RIOK2 promoter and regulate expression. Targeting RIOK2, via newly identified small molecule inhibitors reduces cell viability and soft agar colony growth. Gene set enrichment analysis of RIOK2 depleted PC cells showed reduction of cell cycle, adipogenesis, EMT and cancer stem cell genes. RIOK2 also regulates Neuropeptide Y2 Receptor (NYP2R), which is part of the NPY obesogenic signaling axis that correlates with obesity and worse PC outcomes.

**CONCLUSIONS:** Our in vivo screen highlighted RIOK2 as an actionable PC target in obese hosts. Targeting RIOK2, pharmacologically with our lead compounds or genetically, drastically reduces PC cell viability. RIOK2 may regulate NPY2R expression, which when coupled with elevated NPY in both obese hosts and PCs can amplify NPY protumorigenic signaling.

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