

Regulation of NKX3.1 Protein Stability in Prostate Cancer Cells

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The NKX3.1 gene encodes an androgen-regulated homeodomain transcription factor that functions as a prostate-specific tumor suppressor. The *NKX3.1* locus at 8p21.2 undergoes frequent loss of heterozygosity in prostate cancer, and diminished protein levels are observed in proliferative inflammatory atrophy, prostatic intraepithelial neoplasia, and carcinoma. In cancer, NKX3.1 protein level is inversely correlated with Gleason grade. Discordance between *NKX3.1* mRNA and protein accumulation is observed in a significant proportion of cases, suggesting post-transcriptional or post-translational control. Interestingly, restoration of NKX3.1 protein expression in xenograft and knockout models of prostate cancer suppresses growth.

To shed light on the molecular basis of NKX3.1 turnover in prostate cancer, we have investigated its post-translational control. In-gel kinase assays demonstrated that NKX3.1 is a substrate of Protein Kinase CK2. CK2 inhibition led to a rapid decrease in NKX3.1 accumulation that was reversed by blocking proteasome activity. Mutation of CK2 phosphoacceptor sites diminished NKX3.1 stability. These data demonstrate that CK2 phosphorylation protects NKX3.1 from proteasomal degradation. To further explore the biochemical basis of NKX3.1 stability and turnover, we sought to determine the identity of the ubiquitin ligase activity responsible for marking NKX3.1 for degradation. Two E3 ubiquitin ligases capable of mono- and poly-ubiquitination of NKX3.1 in vitro and in vivo have been identified. Expression of TOPORS and PRAJA1 in LNCaP cells decreased NKX3.1 half-life and led to proteasomal degradation. The PJA1 gene encoding PRAJA1 maps to Xq13.1, within 1.5 megabases of *AR*, and is overexpressed in metastatic prostate cancer based on microarray analyses. These data provide the first insights into the biochemical basis of NKX3.1 turnover in prostate epithelial cells, and identify new targets for therapies designed to restore NKX3.1 expression to inhibit prostate cancer growth and progression. We have recently characterized a C-terminal portable degron that plays a major role in NKX3.1 turnover. Structure-function studies have identified key elements that mediate degron function. These data provide new insights to support the development of peptide therapeutics to restore NKX3.1 expression in prostate cancer.