NKX3.1 stabilizes p53, inhibits AKT activation, and blocks prostate cancer initiation caused by PTEN loss

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Summary

We demonstrate that PTEN loss causes reduced NKX3.1 expression in both murine and human prostate cancers. Restoration of *Nkx3.1* expression in vivo in *Pten* null epithelium leads to decreased cell proliferation, increased cell death, and prevention of tumor initiation. Whereas androgen receptor (AR) positively regulates NKX3.1 expression, NKX3.1 negatively modulates AR transcription and consequently the AR-associated signaling events. Consistent with its tumor suppressor functions, NKX3.1 engages cell cycle and cell death machinery via association with HDAC1, leading to increased p53 acetylation and half-life through MDM2-dependent mechanisms. Importantly, overexpression of *Nkx3.1* has little effect on *Pten* wild-type epithelium, suggesting that PTEN plays a predominant role in PTEN-NKX3.1 interplay. Manipulating NKX3.1 expression may serve as a therapeutic strategy for treating PTEN-deficient prostate cancers.

Introduction

Prostate cancer is the second leading cause of cancer-related death in males (Gregorakis et al., 1998; McDavid et al., 2004). Its development proceeds through a series of defined steps, including prostatic intraepithelial neoplasia (PIN), invasive cancer, and hormone-dependent or -independent metastasis. Although different stages of prostate cancer have been well defined histologically, relatively little is known about the molecular mechanisms contributing to the initiation and progression of prostate cancer.

The *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor gene is frequently mutated in human cancers (Dahia, 2000; Maehama et al., 2001; Parson et al., 1998). The major function of PTEN relies on its phosphatase activity toward PIP3 (phosphatidyl inositol 3,4,5-triphosphate) and, consequently, antagonism of the PI3K (phosphatidylinositol 3-kinase) signaling pathway (Di Cristofano et al., 2001; Maehama et al., 2001). Loss of *PTEN* function results in accumulation of PIP3 and activation of its downstream effectors, such as AKT/PKB (Maehama et al., 2001). AKT, a serine/threonine protein kinase, phosphorylates key intermediate signaling molecules, leading to increased cell metabolism, growth, survival, and invasiveness, all hallmarks of cancer (Di Cristofano et al., 2001; Hanahan and Weinberg, 2000; Vivanco and Sawyers, 2002).

PTEN alteration is strongly implicated in prostate cancer development, as mutations of the *PTEN* gene are found in 30% of primary prostate cancers (Dahia, 2000; Sellers and Sawyers, 2002) and 63% of metastatic prostate tissue samples (Suzuki et al., 1998). Thus, *PTEN* mutations are among the most frequent genetic alterations in human prostate cancer. As PTEN-controlled signaling pathways are frequently altered in human prostate cancers, inhibiting the resultant signaling aberrations will likely serve as promising targets for therapeutic strategies

SIGNIFICANCE

Gene expression profiling of mouse tumor models or human cancers has identified many dysregulated genes that may contribute to tumor development. These wealthy data sets, upon functional validation, may help in elucidating the molecular mechanisms underlying tumorigenesis and providing potential novel targets for cancer therapies. Using a powerful prostate epithelial tissue reconstitution assay, we demonstrated the importance of NKX3.1 in prostate cancer initiation caused by PTEN loss. Our finding emphasizes the cooperative effects between ubiquitously expressed *PTEN* tumor suppressor genes and prostate-specific expressed *NKX3.1* in prostate cancer development. Our study further indicates that validation of candidate genes using mouse models can yield valuable molecular insights that impact human cancer research.

(DeMarzo et al., 2003; Sellers and Sawyers, 2002; Vivanco and Sawyers, 2002).

We and others have developed murine models of prostate cancers by deleting the *Pten* tumor suppressor gene specifically in the prostatic epithelium (Chen et al., 2005; Ma et al., 2005; Trotman et al., 2003; Wang et al., 2003). The *Pten* prostate cancer model recapitulates many features of the disease progression seen in humans with defined kinetics: initiation of prostate cancer with PIN lesions, followed by progression to locally invasive adenocarcinoma, and subsequent metastasis (Wang et al., 2003). Similar to human cancer, *Pten* null murine prostate cancers regress in response to androgen ablation therapy but subsequently relapse and proliferate in the absence of androgens (Wang et al., 2003).

Global assessment of molecular changes caused by homozygous *Pten* deletion identified key genes known to be relevant to human prostate cancer, including those "signature" genes associated with human cancer metastasis (Wang et al., 2003). Among the genes that are downregulated in *Pten* null prostate cancer is *Nkx3.1*, a homeobox gene specifically expressed in the prostate epithelium. NKX3.1 is one of the earliest markers for prostate development and is continuously expressed at all stages during prostate development and in adulthood (Bhatia-Gaur et al., 1999). Human *NKX3.1* maps to chromosome 8p21, a region that frequently undergoes loss of heterozygosity (LOH) at early stages of prostate carcinogenesis (He et al., 1997; Voeller et al., 1997). *Nkx3.1* mutant mice develop prostatic hyperplasia and dysplasia. However, these early lesions failed to progress to metastatic cancers (Abdulkadir et al., 2002; Bhatia-Gaur et al., 1999), consistent with a role for *Nkx3.1* inactivation in prostate cancer initiation.

In this study, we employed a dissociated prostatic epithelial regeneration system to directly test the significance of *Nkx3.1* loss in *Pten* null prostate cancer formation. Our data show that NKX3.1 plays an important role in prostate cancer initiation caused by PTEN loss and that forced *Nkx3.1* expression prevents *Pten* null prostate cancer initiation and progression. Thus, decreased *Nkx3.1* expression contributes to prostate cancer development caused by PTEN loss.

Results

PTEN loss leads to reduced *NKX3.1* expression in both murine and human prostate cancers

Our previous gene expression profiling analysis revealed that *Nkx3.1* mRNA level is downregulated in the *Pten* null prostate cancers (Wang et al., 2003). In this study, consecutive sections of ventral prostate lobe from 4-week-old (4W) *Pten* conditional knockout animals were probed with antibodies to either NKX3.1 or phospho-AKT (P-AKT/Ser 473) (Figure 1A). In the acini where P-AKT levels are low, intense NKX3.1 staining can be observed (Figure 1A, arrows). In contrast, areas with high P-AKT are either low or negative for NKX3.1 staining (Figure 1A, arrowheads). Since increased AKT phosphorylation is a consequence of PTEN loss (Figure S1 in the Supplemental Data available with this article online), these staining patterns suggest that *Nkx3.1* downregulation is an early event linked to *Pten* deletion and prostate cancer initiation.

To test whether PTEN-regulated *Nkx3.1* expression can be observed in human prostate cancers, we conducted double immunofluorescent analysis of PTEN and NKX3.1, using human

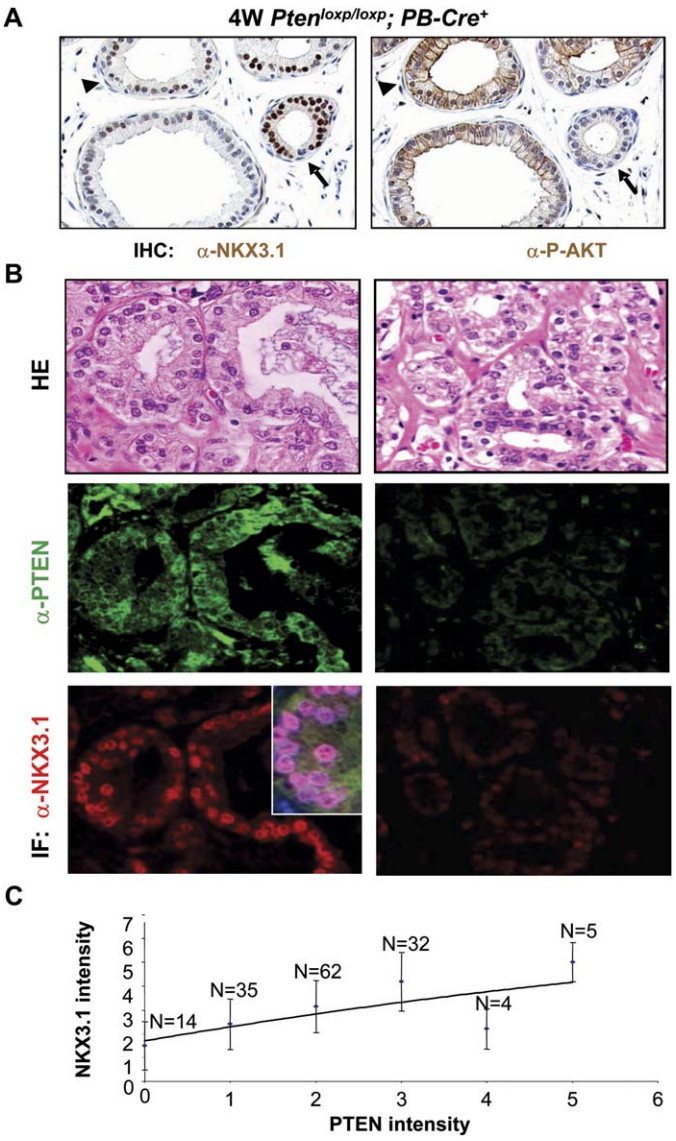

Figure 1. PTEN loss leads to decreased NKX3.1 protein levels in both murine and human prostate epithelium

A: Consecutive sections of 4-week-old *Pten* mutant prostates were probed for NKX3.1 (left) and phospho-AKT (Ser 473) (P-AKT, right) expression. Arrows and arrowheads point to the same duct. Note that NKX3.1 expression reversely correlates with P-AKT staining.

B: High-magnification views of two representative samples from human prostate cancer tissue microarray are shown here. Upper panels: H&E staining; middle and lower panels: double immunofluorescent staining using anti-PTEN (middle) and anti-NKX3.1 (lower) antibodies. Insert: high-power overlay of NKX3.1 and DAPI staining showing NKX3.1 nuclear localization.

C: Correlation of PTEN and NKX3.1 protein levels in 153 human prostate samples. SPSS liner regression was used to analyze data, and the standardized coefficient value was 0.52 ($p < 0.01$ level; $n = 153$).

prostate tissue microarrays (Rocchi et al., 2004). Among 153 samples surveyed (see Experimental Procedures), positive PTEN expression was significantly correlated with NKX3.1 staining, whereas PTEN loss was associated with decreased NKX3.1 staining (Figure 1C; $p < 0.01$). Photos from two representative samples are shown in Figure 1B. Therefore, PTEN loss leads to decreased NKX3.1 expression in both human and murine prostate cancers, implying that NKX3.1 may serve