

Emerging roles of ATF3 in the suppression of prostate cancer

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Stress response mediator activating transcription factor 3 (ATF3) engages in diverse oncogenic pathways including the androgen receptor signaling essential for prostatic proliferation. In line with frequent downregulation of ATF3 expression in human prostate cancers, we have provided the first genetic evidence supporting the role of ATF3 as a tumor suppressor in a subset of prostate cancers with *PTEN* dysfunction.

Activating transcription factor 3 (ATF3) is one of the smallest members of the ATF/CREB family of transcription factors, containing only one functional domain (the basic-region leucine zipper domain, bZIP) that binds the ATF/CREB *cis*-regulatory element (5'-TGACGTCA-3') while dimerizing with other ATF/CREB proteins. The bZIP domain also mediates interactions with many other essential cellular proteins such as p53 (TP53, best known as p53), SMAD family member 3 (Smad3), and mouse double minute 2 (MDM2).¹⁻³ As ATF3 can be rapidly induced by a broad spectrum of cellular stresses (*e.g.*, endoplasmic reticulum stress, oxidative stress, and DNA damage), it is generally believed that it plays important roles in the maintenance of genetic integrity and cellular homeostasis under stressed conditions. Indeed, ATF3 has been shown to engage in diverse cellular signaling pathways, including those mediated by transforming growth factor β (TGF β), Toll-like receptor 4, and the tumor suppressor p53.^{1,2,4} Not surprisingly, recent studies have unveiled crucial but controversial roles of ATF3 in human cancers.⁵ For instance, although ATF3 was previously shown to be a metastasis promoter in a murine B16 melanoma

model, we and others have demonstrated that ATF3 can suppress invasion and metastasis of lung and bladder cancers.^{6,7} In a similar vein, it is reported that ATF3 induces apoptosis and restricts prostate cancer outgrowth while promoting invasion of prostate cancer cells.⁵ Although it is highly possible that ATF3 plays a context-dependent role in cancer, these discrepancies might also reflect intrinsic differences in the cell models that were applied in different studies and that often fail to closely mimic physiological conditions. It is thus important to define the role of ATF3 in cancer using genetically engineered mouse models. In an early study, we examined a *Atf3* knockout (*Atf3*^{-/-}) mouse strain and found that *Atf3* deficiency causes prostatic hyperplasia.⁸ These results are consistent with observations that ATF3 binds the androgen receptor (AR) and represses the androgen signaling that is crucial for sustaining proliferation and survival of prostatic epithelial cells,⁸ and suggest that it is more likely that ATF3 functions as a tumor suppressor for prostate cancer. Indeed, ATF3 expression is frequently downregulated in human prostate cancers.⁸

To provide direct genetic evidence linking ATF3 to prostate cancer suppression,

we crossed the *Atf3*^{-/-} mice (Δ ATF3) with phosphatase and tensin homolog (*Pten*) prostate-specific knockout mice (*Pten*^{Pro(-/-)}, Δ Pten) to generate compound mutant mice (Δ ATF3 Δ Pten).⁹ Inactivation of PTEN by gene mutations or deletion leading to activation of oncogenic phosphoinositide-3-kinase/v-Akt murine thymoma viral oncogene homolog (PI3K/Akt) signaling is one of the most common genetic abnormalities in prostate cancers, occurring in approximately 30% of prostate tumors and up to 60% of metastatic prostate cancers. Interestingly, we found that the oncogenic stress triggered by loss of *Pten* in mouse prostatic epithelium induced expression of both ATF3 and p53, whereas p53 induction was completely abolished in Δ ATF3 mice. These results are consistent with an early report that ATF3 can activate p53 in stressed conditions.² Although p53 activation did not appear to prevent prostate tumorigenesis in our mouse model, we found that the Δ ATF3 Δ Pten mice developed prostatic intraepithelial neoplasia (PIN) and invasive adenocarcinoma at an earlier age and at a higher rate than the Δ Pten mice. Staining for expression of α -smooth muscle actin—an invasion marker—confirmed that the number of invasive glands in the Δ ATF3 Δ Pten mice was significantly increased compared with

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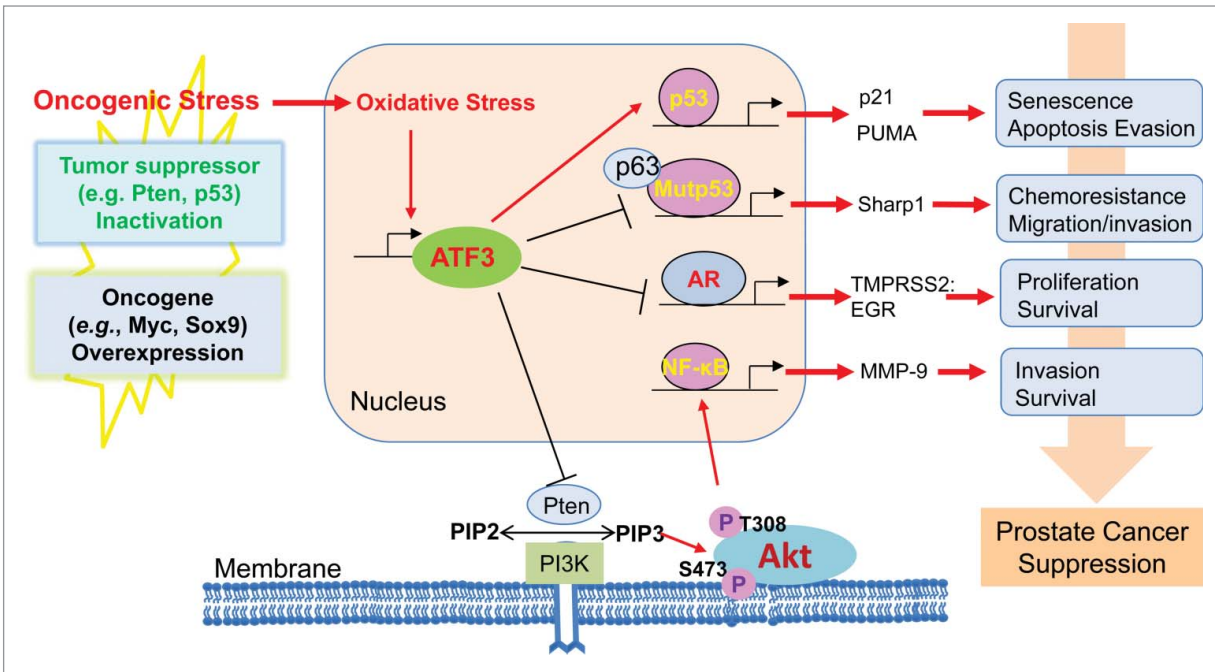


Figure 1. Contribution of activating transcription factor 3 (ATF3) to the suppression of prostate cancer. In addition to regulating the pathways mediated by wild-type and mutant p53 proteins, ATF3 can also suppress androgen receptor signaling while promoting Akt activation in response to the oncogenic stress triggered by inactivation of tumor suppressors and/or overexpression of oncogenes.

that in the $\Delta Pten$ mice. Such effects appeared to be caused by increased proliferation and decreased apoptosis of prostatic epithelial cells. As Akt signaling can increase cell proliferation and promote cell survival, we examined Akt phosphorylation and activation in order to gain further insight into the underlying molecular mechanism by which *Atf3* deficiency promotes prostate cancer development. Whereas loss of *Pten* indeed resulted in Akt activation, as evidenced by elevated Akt Ser473 and Thr308 phosphorylation levels, *Atf3* deficiency dramatically increased Akt phosphorylation levels, indicating that ATF3 suppressed Akt activation in *Pten*-inactivated prostate lesions. To corroborate these important findings, we knocked down *ATF3* expression in LNCaP, PC3, and DU145 human prostate cancer cells using the emerging CRISPR/Cas9 genome editing tool. We confirmed that *ATF3* dysregulation could promote Akt signaling in prostate cancer cells. As Akt signaling is known to activate NF- κ B and regulate expression of matrix metalloproteinases (MMPs) that drive cancer invasion and metastasis, we examined MMP expression and found that expression of MMP-2/MMP-9 was increased in

the ATF3-downregulated human prostate cancer cells as well as in the *Atf3*-deficient mouse prostate lesions. Taken together, results presented in our recently published report⁹ demonstrated that loss of *Atf3* promotes Akt activation and prostate cancer development. Therefore, we have provided the first genetic evidence supporting a role for ATF3 as a tumor suppressor for the major subset of prostate cancers harboring dysfunctional *PTEEN*.

Oncogenic stress triggered by common genetic abnormalities in prostate cancers, such as oncogene overexpression (e.g., Myc and SRY-box 9 [Sox9]) or tumor suppressor inactivation (e.g., PTEN and p53) often generates oxidative stress, which can in turn induce *ATF3* expression (Fig. 1).⁵ Although we previously showed that ATF3 promotes p53 tumor suppressor activity,² we have also demonstrated that ATF3 counteracts chemoresistance and suppresses migration mediated by oncogenic p53 mutations⁶ thereby likely targeting metastatic prostate cancers that often carry p53 mutations. Although this notion remains to be demonstrated, our other studies showed that ATF3 is a physiologically relevant AR repressor that can repress the androgen signaling

indispensable for the growth and survival of prostate cancer cells.⁸ Although ATF3 regulates these cellular functions in the nucleus, our most recent studies have revealed a new role for ATF3 in regulating oncogenic signaling occurring in the cytoplasm (Fig. 1). As ATF3 is predominantly localized in the nucleus of prostate epithelial cells, how this nuclear protein suppresses Akt activation occurring in the cytoplasm remains elusive. It is possible that, as a transcriptional factor, ATF3 alters the expression of one or a set of major Akt regulators such as PH domain and leucine-rich repeat protein phosphatases (PHLPPs) and phosphoinositide-dependent kinase-1 (PDK1). Alternatively, ATF3 might also directly interact with these regulators to modulate their functions. Regardless of the mechanism, results of this recent study combined with our earlier findings suggest that ATF3 induced by oncogenic stress can contribute to suppression of prostate cancer (Fig. 1). It is important to note that recent studies highlight the importance of combined inhibition of PI3K/Akt and AR signaling in the treatment of patients with prostate cancer due to reciprocal feedback regulation of these 2 oncogenic signaling pathways.¹⁰ As ATF3 can suppress both

PI3K/Akt and AR signaling, targeting ATF3 might serve as a promising strategy for therapeutic intervention of prostate cancer.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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