

JNK and PTEN cooperatively control the development of invasive adenocarcinoma of the prostate

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The c-Jun NH₂-terminal kinase (JNK) signal transduction pathway is implicated in cancer, but the role of JNK in tumorigenesis is poorly understood. Here, we demonstrate that the JNK signaling pathway reduces the development of invasive adenocarcinoma in the phosphatase and tensin homolog (*Pten*) conditional deletion model of prostate cancer. Mice with JNK deficiency in the prostate epithelium (ΔJnk $\Delta Pten$ mice) develop androgen-independent metastatic prostate cancer more rapidly than control ($\Delta Pten$) mice. Similarly, prevention of JNK activation in the prostate epithelium ($\Delta Mkk4$ $\Delta Mkk7$ $\Delta Pten$ mice) causes rapid development of invasive adenocarcinoma. We found that JNK signaling defects cause an androgen-independent expansion of the immature progenitor cell population in the primary tumor. The JNK-deficient progenitor cells display increased proliferation and tumorigenic potential compared with progenitor cells from control prostate tumors. These data demonstrate that the JNK and PTEN signaling pathways can cooperate to regulate the progression of prostate neoplasia to invasive adenocarcinoma.

The c-Jun NH₂-terminal kinase (JNK) signaling pathway can target members of the activating protein 1 (AP1) group of transcription factors, including ATF2, c-Jun, JunB, and JunD (1). These transcription factors represent an important component of the immediate-early gene response to mitogens and inflammatory stimuli (2). AP1 transcription factors are also implicated in dysregulated growth and tumor development (2). Significantly, JNK deficiency suppresses AP1-dependent gene expression and causes defects in cell proliferation, senescence, and apoptosis (3–5). JNK may, therefore, play a role in carcinogenesis.

Studies using mouse models of cancer have confirmed that JNK can play a key role in cancer. Thus, JNK deficiency reduces the development of *Bcr/Abl*-induced lymphoma (6) and *KRas*-induced lung tumors (7). Moreover, carcinogen-induced hepatocellular carcinoma (8–10) and skin cancer (11) can be reduced by JNK deficiency. These observations demonstrate that JNK can promote cancer. However, loss of JNK signaling can also promote development of other tumors (2, 9, 12–15). These opposing roles of JNK in tumor development (promotion or repression) may represent differences in JNK function between tumor types (1). Alternatively, these differences may reflect separate functions of JNK in tumor cells and the tumor microenvironment (9).

Mutational inactivation of the tumor suppressor phosphatase and tensin homolog (*PTEN*) frequently occurs in human prostate cancer (16–18). Mouse models of *Pten* deficiency in the prostate epithelium demonstrate that loss of PTEN expression is sufficient to cause activation of the AKT signaling pathway, prostatic intraepithelial neoplasia (PIN) lesions, and subsequent development of castration-resistant prostate cancer (19). Loss of PTEN function is, therefore, established to be a key step in the development of prostate cancer. Importantly, *PTEN* inactivation is associated with increased activity of the JNK signaling pathway in human prostate cancer (20). Indeed, it has been proposed that

JNK may be an effector of the PI3K/AKT pathway in prostate cancer with *PTEN* inactivation (20).

The purpose of this study was to examine the role of the JNK signaling pathway in prostate cancer using a mouse model with selective gene disruption in the prostate epithelium. Previous studies indicate that JNK may be a positive (20) or a negative (21) regulator of prostate cancer development. Here, we report that JNK signaling plays a key role in the development of invasive adenocarcinoma caused by *Pten* inactivation.

Results

JNK Deficiency in the Prostate Epithelium. To test the functional role of JNK, we used a model of prostate cancer using mice with conditional (*floxed*) *Pten* and selective expression of *Cre recombinase* in the prostate epithelium (19). We crossed *Pten* conditional deletion mice to *Jnk1*^{-/-} and *Jnk2*^{-/-} mice on the BALB/cJ strain background. The resulting compound mutant mice developed prostatic neoplastic lesions similar to that of *Pten* single deletion, indicating that JNK1 and JNK2 may be functionally redundant in PTEN-controlled prostate cancer formation. To test this hypothesis, we examined the effect of concomitant deletion of JNK1 plus JNK2 on tumor development. Because *Jnk1*^{-/-} *Jnk2*^{-/-} compound mutant mice die during midembryogenesis (1), we used a conditional (*floxed*) deletion approach. Mice with dual deficiency of *Jnk1* plus *Jnk2* in the prostate epithelium (ΔJnk mice) were viable and fertile. Histopathological analysis of sections prepared from the prostate gland of wild-type (WT) mice and ΔJnk mice indicated that JNK was not required for prostate gland development (Fig. S1).

Mice with triple deficiency of *Jnk1*, *Jnk2*, plus *Pten* (ΔJnk $\Delta Pten$ mice) in the prostate epithelium were also found to be viable and fertile (Fig. 1A). However, the majority of the ΔJnk $\Delta Pten$ male mice died by age 20 wk with large prostate tumors and urethral obstruction (Fig. 1B). In contrast, the mean lifespan of mice with *Pten* deficiency alone ($\Delta Pten$ mice) was greater than 80 wk. Kaplan–Meier analysis demonstrated that the lifespan of ΔJnk

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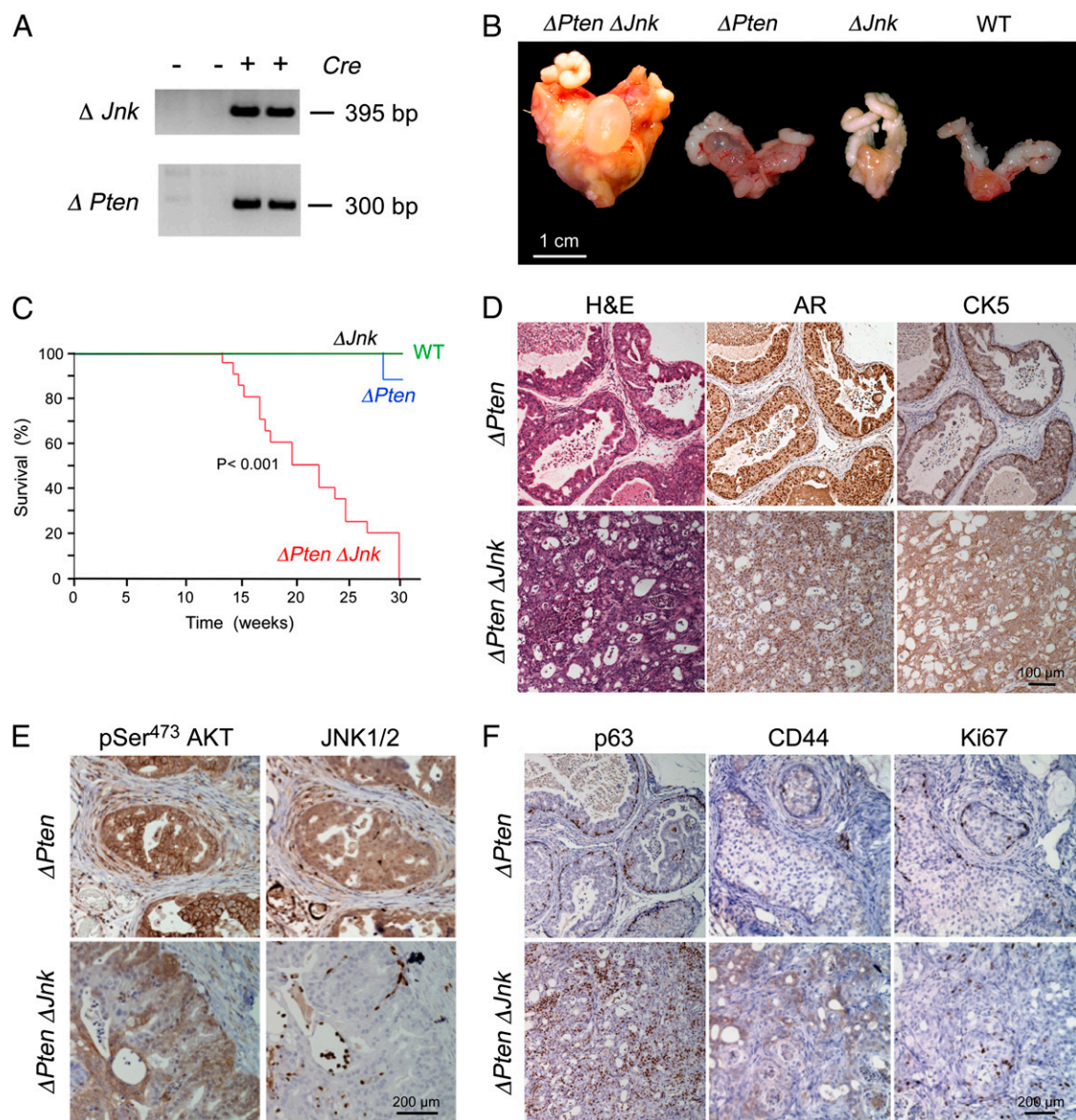


Fig. 1. Loss of JNK cooperates with *Pten* deficiency to promote prostate cancer. (A) Genomic DNA isolated from the anterior prostate gland of $\Delta Pten \Delta Jnk$ mice (Cre^+ and Cre^-) was examined by PCR using amplimers designed to detect the deleted *Pten* and *Jnk* alleles. (B) Representative prostate glands of WT, ΔJnk , $\Delta Pten$, and $\Delta Pten \Delta Jnk$ mice (age 20 wk) are illustrated. (C) Kaplan–Meier analysis of the survival of WT, ΔJnk , $\Delta Pten$, and $\Delta Pten \Delta Jnk$ mice. The lifespan of $\Delta Pten \Delta Jnk$ mice was significantly shorter than $\Delta Pten$ mice ($P < 0.001$). (D) Sections of the anterior prostate of $\Delta Pten$ and $\Delta Pten \Delta Jnk$ mice were stained with H&E or with antibodies to the androgen receptor (AR) or CK5. (E) Sections were stained with antibodies to pSer⁴⁷³ AKT or JNK1/2. (F) Sections were stained with antibodies to p63, CD44, or Ki67.

$\Delta Pten$ mice was significantly shorter than WT mice, ΔJnk mice, or $\Delta Pten$ mice (Fig. 1C). Moreover, the primary tumors present in $\Delta Jnk \Delta Pten$ mice were significantly larger than $\Delta Pten$ mice at age 20 wk (Fig. 1B). Microscopic analysis of sections prepared from these primary tumors demonstrated that the $\Delta Jnk \Delta Pten$ mice displayed significant disruption of prostate glandular structure compared with $\Delta Pten$ tumors (Fig. 1D), consistent with a more advanced tumor phenotype. Indeed, we detected PIN lesions in the primary tumors of $\Delta Pten$ mice and invasive adenocarcinoma in the primary tumors of $\Delta Jnk \Delta Pten$ mice at age 20 wk (Fig. 1D). Immunohistochemical analysis demonstrated the presence of activated AKT in both $\Delta Pten$ and $\Delta Jnk \Delta Pten$ primary tumors, but JNK was detected only in $\Delta Pten$ tumors (Fig. 1E). Nuclear androgen receptors were detected in both $\Delta Pten$ and $\Delta Jnk \Delta Pten$ tumors (Fig. S2).

It is established that JNK can influence proliferation, senescence, and apoptosis (1). The larger prostate tumors in ΔJnk

$\Delta Pten$ mice compared with $\Delta Pten$ mice (Fig. 1) could, therefore, reflect increased growth and/or decreased apoptosis. The majority of cells detected in $\Delta Pten$ PIN lesions expressed senescence-associated β -galactosidase and did not stain for the proliferation marker Ki67 (Fig. S3). It is established that cellular senescence in $\Delta Pten$ PIN lesions limits prostate cancer progression (22, 23). Studies of $\Delta Jnk \Delta Pten$ adenocarcinoma cells demonstrated no expression of senescence-associated β -galactosidase and markedly increased Ki67 staining (Fig. S3). No differences in apoptosis were detected between $\Delta Pten$ and $\Delta Jnk \Delta Pten$ primary tumors (Fig. S3). Together, these data indicate that JNK deficiency in $\Delta Pten$ mice increases prostate cancer development by increasing tumor growth.

Disruption of the JNK Pathway in the Prostate Epithelium. The increased tumor burden in $\Delta Jnk \Delta Pten$ mice compared with $\Delta Pten$ mice (Fig. 1) indicates that JNK may play an important role

during prostate cancer development. However, it is unclear whether these data reflect a role of JNK signaling because functions of nonactivated JNK have been reported (24). To test whether JNK signaling contributes to prostate cancer progression, we examined the effect of deficiency of the MAP kinase kinases (MKK4 and MKK7) that phosphorylate and activate JNK (1). It is established that *Mkk4*^{-/-} and *Mkk7*^{-/-} mice are not viable (1). We, therefore, used conditional alleles to selectively disrupt *Mkk4* and *Mkk7* in the prostate epithelium of $\Delta Pten$ mice (Fig. 2 A–D). This analysis demonstrated similar prostate cancer in $\Delta Mkk4 \Delta Pten$ mice and $\Delta Mkk7 \Delta Pten$ mice. Analysis of tumor sections indicated that the $\Delta Mkk4 \Delta Pten$ and $\Delta Mkk7 \Delta Pten$ neoplastic lesions exhibited greater luminal disruption than $\Delta Pten$ tumors (Fig. 2E), but these tumors did not resemble the invasive carcinoma detected in $\Delta Jnk \Delta Pten$ mice (Fig. 1). MKK4 and MKK7 have partially redundant functions, and complete ablation of the JNK pathway requires compound mutation of both *Mkk4* and *Mkk7* (25). We, therefore, examined prostate tumor development in $\Delta Mkk4 \Delta Mkk7 \Delta Pten$ mice. Compound deficiency of MKK4 plus MKK7 in the prostate epithelium of $\Delta Pten$ mice caused development of invasive adenocarcinoma that was similar to $\Delta Jnk \Delta Pten$ mice (Fig. 2 F–H). The similarity of prostate tumors in $\Delta Jnk \Delta Pten$ mice (Fig. 1) and $\Delta Mkk4 \Delta Mkk7 \Delta Pten$ mice (Fig. 2) indicates that JNK signaling plays an important role in $\Delta Pten$ -dependent prostate carcinogenesis.

Androgen Dependence of JNK-Deficient Prostate Tumors. A hallmark of advanced prostate cancer is the progression to androgen independence (26). Studies of $\Delta Pten$ mice demonstrate that castration (androgen withdrawal) causes dramatic tumor regression followed by the subsequent development of castration-resistant prostate cancer (19). In contrast, castration-induced tumor regression was suppressed in $\Delta Jnk \Delta Pten$ mice compared with $\Delta Pten$ mice (Fig. S4). The $\Delta Jnk \Delta Pten$ tumors from castrated mice exhibited increased glandular disruption, decreased E-cadherin expression, and disorganized expression of α -smooth muscle actin compared with $\Delta Jnk \Delta Pten$ tumors (Fig. S5). The aggressive prostate cancers detected in $\Delta Jnk \Delta Pten$ mice therefore markedly differ from tumors detected in $\Delta Pten$ mice in their response to androgen withdrawal. This observation is consistent with a more advanced tumor phenotype in $\Delta Jnk \Delta Pten$ mice compared with $\Delta Pten$ mice.

JNK and Prostate Tumor Metastasis. Pathological examination of $\Delta Jnk \Delta Pten$ mice indicated the presence of metastatic cells at sites distant from the primary tumor. The lumbar and caudal lymph nodes are established to be preferential sites for metastasis in orthotopic mouse models of prostate cancer (27, 28). Metastasis of $\Delta Pten$ prostate tumor cells to these lymph nodes has been reported (29). We found that the lumbar lymph nodes of $\Delta Jnk \Delta Pten$ mice were enlarged compared with $\Delta Pten$ mice at age 20 wk (Fig. 3A). Androgen-receptor-positive metastatic cells in the lymph

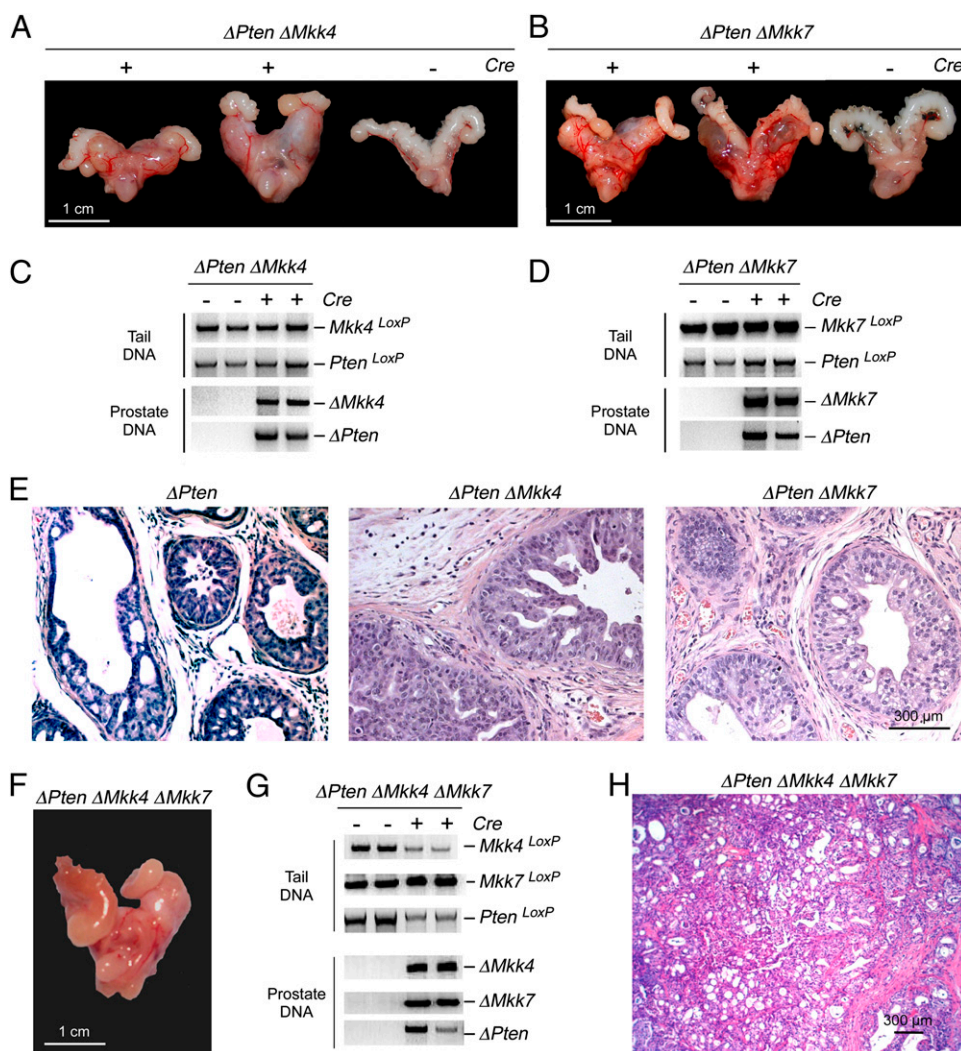


Fig. 2. Effect of *Mkk4* and *Mkk7* gene ablation on $\Delta Pten$ -dependent prostate cancer. (A and B) Representative images of prostate glands from $\Delta Mkk4 \Delta Pten$ and $\Delta Mkk7 \Delta Pten$ mice (age 20 wk) are illustrated. (C and D) Genomic DNA isolated from the prostate gland and tail of $\Delta Mkk4 \Delta Pten$ (C) and $\Delta Mkk7 \Delta Pten$ mice (D) were genotyped for *Mkk4*, *Mkk7*, and *Pten* alleles. (E) Representative H&E-stained tissue sections of the anterior prostate glands of $\Delta Pten$ mice, $\Delta Mkk4 \Delta Pten$ mice, and $\Delta Mkk7 \Delta Pten$ mice (age 20 wk) are presented. (F–H) Representative image of a $\Delta Mkk4 \Delta Mkk7 \Delta Pten$ prostate gland (20-wk-old mouse) is illustrated (F). Genomic DNA isolated from the prostate gland and tail of $\Delta Mkk4 \Delta Mkk7 \Delta Pten$ mice were genotyped for *Mkk4*, *Mkk7*, and *Pten* alleles (G). A representative H&E-stained tissue section prepared from the anterior prostate gland of a $\Delta Mkk4 \Delta Mkk7 \Delta Pten$ mouse (20 wk old) is presented (H).

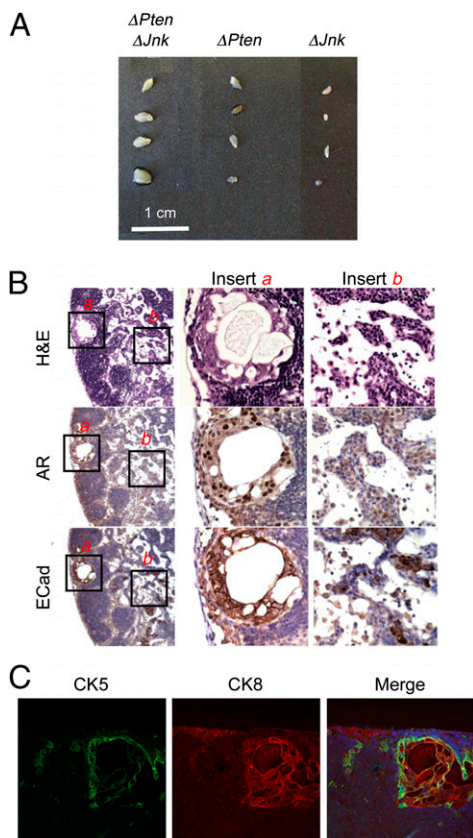


Fig. 3. Loss of JNK promotes prostate tumor metastasis. (A) Lumbar lymph nodes isolated from ΔJnk , $\Delta Pten$, and $\Delta Pten \Delta Jnk$ mice (age, 20 wk) are illustrated. (B) Representative sections of $\Delta Pten \Delta Jnk$ lumbar lymph nodes were stained with H&E or with antibodies to the androgen receptor (AR) or E-cadherin (ECad). (C) Representative sections of $\Delta Pten \Delta Jnk$ lumbar lymph nodes were stained with antibodies to CK5 and CK8. The merged image includes the DNA stain DAPI.

nodes of $\Delta Jnk \Delta Pten$ mice were detected (Fig. 3B). These metastatic cells formed organized duct-like structures with basal expression of cyokeratin (CK)5 and luminal expression of CK8 that resemble prostate epithelium (Fig. 3C). In contrast, metastasis was not detected in $\Delta Pten$ mice (Fig. 3). The failure to detect metastasis in $\Delta Pten$ mice is consistent with previous observations (23) but differs from the low incidence of metastasis detected in studies of $\Delta Pten$ mice on a mixed genetic background (19). Together, these observations are consistent with the conclusion that $\Delta Jnk \Delta Pten$ mice rapidly develop advanced prostate cancer.

JNK Regulates the Tumorigenic Potential of $\Delta Pten$ Prostate Tumor Cells. It is established that ablation of *Pten* causes an increase in the immature cell compartment within the prostate gland (30). However, the prostate glands of $\Delta Jnk \Delta Pten$ mice were found to contain a much larger population of immature prostate cells that stained with antibodies to p63 and cluster of differentiation (CD) 44 compared with $\Delta Pten$ mice at age 20 wk (Fig. 1F). This expansion of immature prostate cells was also detected in castrated mice (Fig. S6) and is, therefore, androgen-independent.

The expansion of the immature cell population in the primary prostate tumors of $\Delta Jnk \Delta Pten$ mice compared with $\Delta Pten$ mice is intriguing. To test whether these cells contribute to the tumor phenotype, we isolated lineage-negative (Lin^-) $Sca1^+$ cells from $\Delta Jnk \Delta Pten$ and $\Delta Pten$ primary tumors and cultured these cells in vitro. Previous studies using WT mice have demonstrated that this procedure leads to the formation of spheres (prostatespheres)

with a surface location of prostate stem cells and a luminal location of more differentiated cells (31). Luminal prostate stem cells may also be present in these cultures (32, 33). Comparison of $\Delta Jnk \Delta Pten$ and $\Delta Pten$ prostaspheres demonstrated that JNK deficiency caused a significant increase in growth during culture in vitro (Fig. 4A). Microscopic analysis of sections prepared from $\Delta Jnk \Delta Pten$ and $\Delta Pten$ prostaspheres indicated that JNK deficiency caused altered morphology, including reduced luminal cell–cell interactions (Fig. 4B) and increased luminal cell apoptosis (Fig. S7). Immunofluorescence analysis demonstrated strong staining of immature progenitor cell markers (p63, CK5, nucleostemin, and integrin $\alpha 6$) in a distinct surface zone of $\Delta Jnk \Delta Pten$ prostaspheres compared with $\Delta Pten$ prostaspheres (Fig. 4C and D and Fig. S8).

The tumorigenic potential of the $Lin^- Sca1^+$ cells isolated from $\Delta Jnk \Delta Pten$ and $\Delta Pten$ prostate tumors was examined using renal capsule transplantation assays with immunodeficient host mice (Fig. S9). No growth was detected in studies using $Lin^- Sca1^+ \Delta Pten$ cells, a population of cells that includes the tumor-initiating cells of the $\Delta Pten$ prostate cancer model (34). In

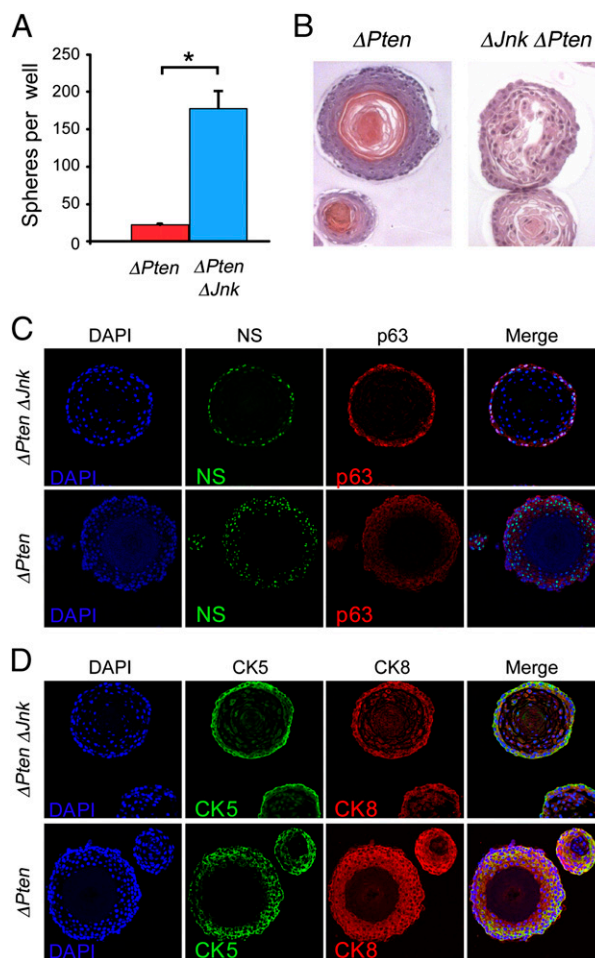


Fig. 4. Loss of JNK promotes proliferation of immature $\Delta Pten$ prostate cells. (A) The cloning efficiency of $Sca1^+$ cells isolated from $\Delta Pten$ and $\Delta Pten \Delta Jnk$ prostate tumors was measured at passage 2 in vitro. Equal numbers of $Sca1^+$ cells were plated and the number of prostaspheres obtained after 14 d in culture was examined (mean \pm SD; $n = 3$). Significant differences are indicated ($*P < 0.05$). (B) Sections of prostaspheres were stained for DNA (DAPI) and the stem cell markers p63 and nucleostemin (NS). (C and D) Sections of prostaspheres were stained with H&E or with DNA (DAPI) plus basal (CK5) and luminal (CK8) differentiation markers.

contrast, *Lin⁻ Sca1⁺ ΔJnk ΔPten* cells efficiently formed kidney capsule tumors (Fig. S9).

Discussion

JNK Is Not Required for the Development of Prostate Neoplasia. Two protein kinases (MKK4 and MKK7) that phosphorylate and activate JNK (1) are detected in benign human prostate epithelial cells (35). Studies of human prostate cancer demonstrate that the expression of MKK4 and MKK7 (but not JNK) is increased in PIN lesions (35). Signaling by the JNK pathway may, therefore, be increased during the formation of PIN lesions. Indeed, *PTEN* inactivation in human prostate tumors is associated with increased JNK activity (20). Together, these data indicate that JNK may promote the formation of PIN lesions and may function as an effector of increased PI3K/AKT signaling caused by *PTEN* inactivation (20). To test this prediction, we used a mouse model with conditional deletion of *Pten* in the prostate epithelium. This analysis demonstrated that JNK in prostate epithelial cells is not essential for the formation of neoplastic lesions. However, it remains possible that JNK in stromal cells that support epithelial cell morphogenesis and differentiation (36) may play a role in the development of PIN lesions. Further studies will be required to test this hypothesis in the context of prostate cancer, but recent studies have established that JNK in stromal cells can promote carcinogenesis (9). Nevertheless, JNK in prostate epithelial cells is not required for prostate cancer development caused by *Pten* inactivation in mice.

JNK Regulates Cancer Progression to Invasive Adenocarcinoma. Although JNK in the prostate epithelium is not essential for the formation of neoplastic lesions in the conditional *Pten* deletion mouse model, it is possible that JNK may contribute to PIN lesion maintenance by contributing to the cellular senescence program that limits prostate cancer progression (22, 23). Indeed, the senescence of primary $\Delta Pten$ PIN lesions was not detected in the prostate glands of $\Delta Jnk \Delta Pten$ mice. These data indicate that JNK signaling may maintain PIN lesions by reducing growth and inducing senescence and that loss of JNK signaling promotes the development of invasive adenocarcinoma.

The concept that JNK may restrain progression to adenocarcinoma is consistent with the observation that MKK4 is down-regulated in advanced stage human prostate cancer (21). The mechanism of down-regulation is caused, in part, by translation inhibition (37) that may be mediated by microRNA pathways, including miR15b, miR-24, miR-25, and miR-141 (38). Intriguingly, increased MKK4 caused by decreased microRNA expression is associated with senescence (38). Together, these data indicate that decreased JNK signaling in PIN lesions may contribute to cancer progression and the formation of adenocarcinoma.

The *MKK4* gene has been identified previously as a putative human metastasis suppressor in prostate cancer (21, 39–41) and other forms of cancer (42–44). Moreover, mutations in JNK pathway genes (*MKK4*, *MKK7*, *JNK1*, and *JNK2*) have also been identified in human prostate cancer (45–47). A functional role for *MKK4* deficiency is consistent with the finding that decreased JNK signaling in the $\Delta Pten$ mouse model promotes the development of invasive adenocarcinoma. However, the effect of *MKK4* or *MKK7* deficiency on murine prostate cancer was reduced compared with JNK deficiency (Figs. 1 and 2). This observation is consistent with previous reports that deficiency of *MKK4* or *MKK7* reduces JNK activity (25). Compound deficiency of *MKK4* plus *MKK7* is required to ablate JNK signaling (25) and to fully promote adenocarcinoma development (Fig. 2). Additional mechanisms may therefore contribute to the proposed effects of *MKK4* deficiency in humans (41). Thus, the dominant-negative activity of catalytically inactive *MKK4* proteins (caused by cancer-associated *MKK4* gene mutations) may cause greater suppression of JNK activity than *MKK4* gene

ablation (42, 45). Moreover, *MKK4* gene mutation in human prostate cancer may cooperate with other genetic alterations (e.g., increased *DUSP1* expression) that suppress JNK activity (48).

JNK and PTEN Cooperate to Regulate the Development of Invasive Adenocarcinoma. It is established that *PTEN* inactivation is an important event in the development of human prostate cancer. Loss of *PTEN* in the prostate epithelium causes activation of the PI3K/AKT pathway (19) and inhibition of androgen receptor signaling (49, 50). Reciprocal regulation of PI3K/AKT and androgen receptor signaling represents a mechanism of signaling cooperation that regulates prostate cancer development. Similarly, dysregulated *Smad4* (23), *Erg* (51, 52), *cMyc* (53), and *Trp53* (22) cooperate with *PTEN*/AKT to promote prostate cancer. The results of this study identify JNK as another signaling pathway that cooperates with *PTEN* deficiency to regulate cancer progression in the prostate gland. The effects of JNK deficiency to increase both tumor size and invasive adenocarcinoma/metastasis more closely resembles dysregulated *Smad4* than dysregulated *cMyc*, *Erg*, or *Trp53*. This observation indicates that crosstalk between JNK and TGF- β signaling (54) may contribute to tumor progression. *PTEN* deficiency therefore promotes carcinogenesis by altering the cell signaling network. Perturbation of this signaling network can promote or repress tumorigenesis. In this context, JNK cooperates with *PTEN* to regulate progression to invasive adenocarcinoma.

Compound JNK deficiency in epithelial cells of the prostate (this study), breast (2), and liver (9) causes an increase in carcinogenesis. This observation suggests that JNK may act to reduce tumor development in these epithelial tissues.

Materials and Methods

Mice. Mice with ablation of the *Jnk1* (55) and *Jnk2* (56) genes and also mice with conditional (*floxed*) alleles of *Jnk1* (5), *Mkk4* (57), and *Pten* (58) have been described. BALB/cJ and NOD.Cg-Prkdc^{cid}/Il2rg^{tm1Wjl}/SzJ mice were obtained from The Jackson Laboratory. Mice with *floxed* alleles of *Mkk7* were created using homologous recombination in embryonic stem cells, blastocyst injection of E5 cells to create chimeric mice, and breeding to obtain germ-line transmission of the mutated *Mkk7* allele with *LoxP* sites inserted in intron 3 and intron 7. *PB-Cre4* mice were provided by Dr. P. Roy-Burman (59). These mice were back-crossed (ten generations) to the BALB/cJ strain background. These mice were crossed to obtain compound mutants: $\Delta Pten$ (*PB-Cre4⁺ Pten^{LoxP/LoxP}*); ΔJnk (*PB-Cre4⁺ Jnk1^{LoxP/LoxP} Jnk2^{-/-}*); $\Delta Jnk \Delta Pten$ (*PB-Cre4⁺ Pten^{LoxP/LoxP} Jnk1^{LoxP/LoxP} Jnk2^{-/-}*); $\Delta Mkk4 \Delta Pten$ (*PB-Cre4⁺ Pten^{LoxP/LoxP} Mkk4^{LoxP/LoxP}*); $\Delta Mkk7 \Delta Pten$ (*PB-Cre4⁺ Pten^{LoxP/LoxP} Mkk7^{LoxP/LoxP}*); and $\Delta Mkk4 \Delta Mkk7 \Delta Pten$ (*PB-Cre4⁺ Pten^{LoxP/LoxP} Mkk4^{LoxP/LoxP} Mkk7^{LoxP/LoxP}*). Studies of $\Delta Pten$ mice demonstrated that tumor development in BALB/cJ mice was similar to previous reports using mixed strain background mice (19), although progression to PIN lesions and invasive adenocarcinoma was slower in the BALB/cJ strain background. Mice were castrated using a surgical procedure (19). The animal studies were approved by the Institutional Animal Care and Use Committees of the University of Massachusetts Medical School and the University of California, Los Angeles. The mice were housed in facilities approved by the American Association of Laboratory Animal Care (AALAC).

Analysis of Tissue Sections. Histology was performed using tissue fixed in 10% (vol/vol) formalin for 24 h, dehydrated, and embedded in paraffin. Sections (7 μ m) were cut and stained using hematoxylin and eosin (H&E) (American Master Tech Scientific). Apoptotic cells were detected using the ApopTag reagent (Millipore). Immunohistochemistry was performed by staining with a primary antibody [androgen receptor (Santa Cruz; sc-816), CK5 (Covance; PRB-160), CD44 (eBioscience; 14-004), E-cadherin (BD Transduction; 610181), P-AKT-Ser473 (Cell Signaling; 3787), JNK1/2 (BD Pharmingen; 554285), smooth muscle actin (Sigma; A5228), and K167 (Vector; VP-RM04)], a biotinylated secondary antibody (Biogenex), streptavidin-conjugated horseradish peroxidase (Biogenex), and the substrate 3,3'-diaminobenzidine (Vector Laboratories), followed by brief counter staining with Mayer's hematoxylin (Sigma). Immunofluorescence analysis was performed by staining with antibodies to CK5 (Covance; PRB-160) and CK8 (Covance; MMS-162) and detection using fluorescence-conjugated secondary antibodies (Invitrogen). Fluorescent images were examined using a fluorescence microscope.

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