Function of JunB in Transient Amplifying Cell Senescence and Progression of Human Prostate Cancer

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Abstract Purpose: Replicative senescence in cells acts as a barrier against excessive proliferation and carcinogenesis. Transient amplifying cells (TAC) are a subset of basal cell populations within the prostate from which cancers are thought to originate; therefore, we focused on prostate TAC to investigate the molecular mechanisms by which the TAC may be able to evade senescence. Experimental Design: TAC clones were isolated from each zone within the whole prostate and analyzed in flow cytometry. Prostate cancer cells were transfected with junB small interfering RNA (siRNA) and examined by chorioallantoic membrane assay for cancer invasion. Immunohistochemical analysis was done in primary and metastatic prostate cancer specimens. Results: TAC populations showed increased expression of p53, p21, p16, and pRb, resulting in senescence. TAC clones with reduced p16 expression successfully bypassed this phase. We further found close correlation between the levels of junB and p16 expression. Repeated transfection of junB siRNA in prostatic TAC allowed the cells to escape senescence presumably through inactivation of p16/pRb. The chorioallantoic membrane invasion assay showed much lower in invasive cancer cells with high expression of junB; conversely, silencing of junB by transfection with junB siRNA promoted invasion. We also found that metastatic prostate cancers, as well as cancers with high Gleason scores, showed significantly low junB immunopositivity. Conclusions: JunB is an essential upstream regulator of p16 and contributes to maintain cell senescence that blocks malignant transformation of TAC. JunB thus apparently plays an important role in controlling prostate carcinogenesis and may be a new target for cancer prevention and therapy.

Normal prostate epithelium is composed of luminal, basal, and neuroendocrine cells with a balance of proliferation such that neither involution nor hyperplasia of the glands occurs (1). Within the basal cell population, there are two cell types: stem cells and transient amplifying cells (TAC). Stem cells have the capacity to differentiate into TAC, which then mature into intermediate cells that migrate into the luminal layers (2, 3). Several in vivo and in vitro studies have recently focused on stem cells/TAC as the origin of prostate cancer (3, 4). This cancer stem cell hypothesis consists of two major postulates: (a) tumors are derived from organ stem cells that acquire epigenetic/genetic changes, leading to transformation, and (b) cancer stem cells are synthesized de novo within tumors and acquire a self-renewing capacity, which might be involved in initiation and/or promotion of human neoplasias. In addition, these cancer stem cells have been suggested to be resistant to conventional chemotherapies (5, 6), which, therefore, are unlikely to be curative, although they may eradicate the majority of cancer cells arising from non-stem cell populations. Although debate remains as to the validity of stem cell theories in carcinogenesis in general, investigations of the major molecular mechanisms involved in the regulation of prostate stem cells may provide valuable insights into prostatic carcinogenesis and the development of more efficacious therapeutics. For instance, both stem cells and TAC are unresponsive to androgen (7), which may explain the emergence of androgen independence. Growing numbers of reports relating to prostatic stem cells have been accumulating; however, there is little data, to date, on the role of prostate TAC.

In most cancers, including prostate, multiple genetic and epigenetic alternations are accumulated before malignant transformation occurs (8, 9). Immortalization is one of the major steps in carcinogenesis that is vitally important for the continuing evolution and progression of most advanced cancers, and immortalization is primarily prevented by replicative senescence (10, 11). Cell cycle checkpoint pathways are activated and the cell cycle is arrested in the senescent state (12, 13). If, however, the cells can avoid or reverse such pathways, they can bypass the senescence-associated cell cycle arrest and continue to proliferate beyond their normal lifespan,
resulting in tumorgenesis (14, 15). There are telomere-dependent and telomere-independent mechanisms by which replicative senescence occurs. Telomere shortening is known to trigger senescence via a pathway involving ataxia telangiectasia mutated kinase, the tumor suppressor p53, and the cyclin-dependent kinase inhibitor p21 (16, 17). In contrast, telomere-independent senescence appears to involve cyclin-dependent kinase inhibitor p16 and the retinoblastoma tumor suppressor as the terminal effectors (18). In murine cells, pRb and p53 are required not only for the onset but also for maintenance of senescence, and inactivation of p53/pRb can lead to senescence reversal. In human cells, on the other hand, once p16/pRb is fully engaged and senescence occurs, replicative capacity cannot be retrieved even if p16/pRb signals are subsequently inactivated (19–21). It remains unclear whether there are any mechanisms, in fact, by which human cells can revoke growth arrest following p16/pRb-mediated cellular senescence.

Biological behavior is clinically different among prostate cancers arising from the central zone (CZ), transition zone (TZ), and peripheral zone (PZ) of the gland. Most predominant and aggressive cancers occur in the PZ, with less aggressive cancers and benign prostatic hyperplasia occurring exclusively in the TZ and much less in the CZ. This suggests different molecular mechanisms based on distinct and specific genetic alterations (22, 23). Therefore, examination of TAC derived from the biologically different zones may give insights into the mechanisms underlying prostate carcinogenesis. In the present study, we investigated the molecular mechanisms by which TAC derived from human prostatic tissue can bypass senescence and the role of gene alterations associated with senescence in prostate cancer progression. We found that, in human prostatic TAC, junB has a novel upstream signal function leading to the p16/pRb activation essential for initiation and maintenance of senescence. In addition, in vitro and in vivo assays, as well as immunohistochemical analyses, on tumor samples obtained from radical prostatectomies clearly showed that activated junB suppresses malignant transformation of prostate cancer as measured by invasion and metastasis.

Materials and Methods

Isolation of normal TAC fractions from human prostatic tissue. We obtained prostate tissues from eight patients (age range: 54-73 years) undergoing radical prostatectomy without radiotherapy, hormonal therapy, and chemotherapy. All patients provided informed consent before collection of specimens. Within these excised tissues, all cancers identified and confirmed by pathologic analysis were confined to one hemisphere of each gland, allowing the normal contralateral side to be a source of tissue for isolation of prostate-derived TAC.

Because TAC characteristically show high expression of α3β1-integrin, we isolated those populations of cells highly expressing α3β1 according to the methods detailed in previous reports (24). Briefly, those portions of the eight harvested prostate glands, diagnosed by two pathologists to be free of invasive carcinoma, were divided into CZ, TZ, and PZ and digested with collagenase. The glandular and acinar components were subsequently separated from the stromal fraction by repeated centrifugation, yielding epithelial populations of >95% purity (data not shown). Purified acinar cells were further treated with 0.25% trypsin/EDTA (Life Technologies), 0.01% DNase I, 4 mmol/L MgCl2, and 10 mmol/L HEPES (Life Technologies) for 30 min at 37°C to produce single-cell suspensions. Luminal cells were removed by passing the cell suspensions through columns of anti-CD57-linked beads (Invitrogen). Eluted basal cells were further fractionated based on adhesion to type I collagen for 5 min (BD Biosciences) to obtain those with high α3β1-integrin expression. These cells were incubated with MACS microbeads linked to anti-CD133 for 1 h before washing twice in MACS buffer followed by centrifugation and elution through a magnetic MACS mini-column. The column was washed and the eluted α3β1/CD133 cells were collected and incubated in a low-calcium (<300 μmol/L) medium containing bovine pituitary extract and recombinant epidermal growth factor (Cambrex). We were able to obtain TAC clones specifically from CZ, TZ, and PZ of each prostate in this fashion. These clones were propagated in culture for various lengths of time for immunohistochemical flow cytometry, transfection, and protein expression analyses over time.

Primary and metastatic prostate tumor samples and immunohistochemistry. Radical prostatectomies done at Nara Medical University provided samples of an additional 55 primary prostate carcinomas and 7 metastatic cases (3 lung, 2 bone marrow, 1 liver, and 1 kidney) for our junB immunohistochemical analyses. All tumor samples came from patients without previous chemotherapy, and the study was approved by the Ethics Committee of Nara Medical University School of Medicine. Tissue fixation and processing followed that described in a previous report (8, 25), and H&E slides were pathologist reviewed for tumor staging and scoring according to the Gleason system for prostate carcinomas (26, 27). Mounted sections were incubated with the appropriate primary antibodies for 16 h at 4°C and the reactions visualized using a Histofine SAB-PO kit (Nichirei), with diaminobenzidine as the chromogen and light hematoxylin counterstaining. The degree of immunopositivity was expressed as the percentage of positive cells in at least 1,000 cells examined.

Human prostate cancer cell line culture. We purchased the human prostate cancer cell line, DU145 (American Type Culture Collection), and cultured the line to confluence in RPMI supplemented with 10% fetal bovine serum. We were able to obtain cells with various degrees of junB protein expression by subcloning those cells escaping confluence-induced cell death in the DU145 cultures. Anti-p53, anti-p21, anti-p16, anti-actin, and anti-green fluorescence protein (GFP) were purchased from Santa Cruz Biotechnology. Anti-cytokeratin 5 came from Novocastra, whereas anti-retinoblastoma was from BD Biosciences. Anti-p63, anti-α3β1 integrin, and anti-androgen receptor were from DAKO. Anti-junB was purchased from Abcam (ab31421).

Immunocytochemistry. We seeded TAC from CZ, TZ, and PZ clones onto collagen I–coated tissue culture slides (BD Biosciences), fixed them in methanol at -20°C, and air dried the slides. Following blocking with 20% blocking serum, the slide-mounted cells were incubated with antibodies against CD133, cytokeratin 5, p63, or androgen receptor for 1 h at 37°C, and the antigenic reactions were visualized using a Histofine SAB-PO kit (Nichirei) as described above. For β-galactosidase staining, we fixed the cells with 2% formaldehyde and 0.2% glutaraldehyde in PBS (pH 6.0) for 5 min at 4°C. After washing, the cells were overlaid with X-gal staining solution (5 mmol/L potassium ferricyanide, 5 mmol/L potassium ferrocyanide, 1 mmol/L MgCl2, 0.02% NP-40, and 1 mg/mL X-gal in PBS) for 1 h at 37°C in the dark.

Flow cytometry. Cells from purified TAC were analyzed in flow cytometry as described previously (28). Briefly, 1 × 10⁶ cells were washed with 1 mmol/L EDTA-PBS followed by two washes with flow cytometric buffer containing PBS (pH 7.4), 1% FCS, and 0.01% sodium azide. They were then resuspended in the same buffer and incubated with mouse anti-α3β1-integrin monoclonal antibody or anti-mouse IgG1 as negative control at 4°C for 1 h. After washing twice, the cells were incubated with FITC-conjugated rabbit anti-mouse IgG (DAKO) in the dark again at 4°C for 1 h followed by an additional double wash.

Preparation of cell lysates and immunoblotting analysis. Immunoblotting using cell lysates was conducted as described previously (29, 30). Briefly, we washed the cells with PBS and suspended them in lysis buffer (40 mmol/L HEPES (pH 7.4) with 10% glycerol, 1% Triton X-100, 0.5% NP-40, 150 mmol/L NaCl, 50 mmol/L NaF, 20 mmol/L β-glycerophosphate, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L phenylmethylsulfonyl fluoride, and 0.1 mmol/L vanadate) containing a

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protease inhibitor mixture (aprotinin, leupeptin, and pepstatin). Cells lysates were cleared by centrifugation at 15,000 rpm for 30 min and then resolved on SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore). The membranes were blocked in TBST buffer [20 mmol/L Tris-HCl (pH 7.5) containing 150 mmol/L NaCl and 0.1% Tween 20] with 5% skim milk at room temperature for 1 h and then incubated with indicated primary antibodies for 16 h at 4 °C. After washing with TBST, the membranes were incubated with horseradish peroxidase–conjugated anti-mouse or anti-sheep IgG (Amersham Pharmacia Biotech). After further washing with TBST, we used an enhanced chemiluminescence detection system to detect peroxidase activity.

Reverse transcription-PCR. Using the One-step Reverse Transcription-PCR kit (manufacturer’s protocol; Qiagen), we extracted total RNA using Trizol reagent and subjected it to reverse transcription-PCR. PCR conditions were 94°C for 30 s, 55°C to 60°C for 30 s, and 72°C for 1 min through a total of 35 cycles. The PCR primer sequences for junB were 5'-ACTCATACACAGCTACGGGATACG-3' (sense) and 5'-GGCTCGGTTTCAGGAGTTTG-3' (antisense). The primers for matrix metalloproteinase (MMP) 2 were 5'-ATGACAGCTGCACCACTGAG-3' (sense) and 5'-ATTTGTTGCCCAGGAAAGTG-3' (antisense). The primers for glyceraldehyde-3-phosphate dehydrogenase used were 5'-ACCA-CACCAGTCCATGCCATCAC-3' (sense) and 5'-TCCACCACCCTGT-TGCTGTA-3' (antisense). PCR products were analyzed on a 1.5% agarose gel and visualized by ethidium bromide staining.

Transfection of junB small interfering RNA. We seeded 10⁶ DU145 cells per well in 6-cm culture plates and transfected them with either 100 nmol/L control RNA or junB interfering RNA (siRNA; Santa Cruz Biotechnology) in the presence of a plasmid vector carrying GFP (pEGFP; Clontech) using Lipofectamine (Invitrogen) in accordance with the manufacturer’s protocol. After cultivation for the indicated time, we seeded the transfected cells onto chick chorioallantoic membranes (CAM) and incubated them for 3 days. At the end of the incubation period, we removed, homogenized, and lysed the underlying CAM tissues. The CAM lysates were then analyzed for junB expression by immunoblotting and reverse transcription-PCR.

Chick CAM invasion assay. To investigate the potential for invasion and intravasation of DU145 cells in vivo, we again employed the CAM assay. This time, we seeded 10⁵ GFP-transfected cells (16 h transfection with pEGFP) onto 11-day-old CAM for 3 days (31). After the 3-day incubation period, CAM tissues were fixed and stained with anti-GFP and the numbers of GFP-positive cells invading the membranes were quantified in three or more randomly selected microscopic fields. The depth of invasion from the CAM surface was also quantified as the leading front of the three or more invading cells in five randomly selected fields. We did each CAM assay in triplicate or more.

Cell viability assays. We seeded DU145 cells into 96-well plates and treated them with either hydroxyurea or nocodazole for 24 h followed by stimulation with 25 mg/mL etoposide for an additional 48 h. We then added the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt reagent (Promega) to each well. After 3-h incubation period, we measured the absorbance at 490 nm using a microplate reader. Cell viability was expressed as the mean ± SE absorbance at 0, 24, and 48 h of incubation. All experiments were done in triplicate.
Isolation and culture of TAC. Within basal cell population expressing high levels of $\alpha_3\beta_1$-integrin, Collins et al. (24) have further found that TAC populations can be identified from stem cells based on CD133 status (the TAC is CD133$^+$, whereas stem cells are CD133$^-$). As shown in Fig. 1A and B, we were able to isolate and culture cells expressing high levels of $\alpha_3\beta_1$-integrin that are also positive for p63 and cytokeratin 5 and negative for CD133 and androgen receptor, which corroborate with more recently reported characteristics of the TAC (33). We also confirm culture conditions (serum-free medium with >700 $\mu$mol/L calcium) for TAC from all three prostatic morphologic zones as well as for a stem cell subset of intermediate quiescent cells that do not fully mature into luminal secretory cells (34). Following five serial passages, we obtained 65, 82, and 52 clones from CZ, TZ, and PZ, respectively. They were all proliferating until they reached confluence. Confluent culture may damage cells, creating more avenues for growth selection. To obtain multiple malignant phenotypes, we cultured an androgen-independent prostate cancer cell line, DU145, and maintained them in stationary avenues for growth selection. To obtain multiple malignant phenotypes, we cultured an androgen-independent prostate cancer cell line, DU145, and maintained them in stationary

JunB and p16/pRb signals in bypass of senescence. We selected four clones from each zone (C1, C2, C3, and C4; T1, T2, T5, and T6; and P2, P3, P4, and P6) to examine expression of p53, p21, p16, and pRb in senescence. As shown in Fig. 2 (left), p53, p21, and p16 expression increased in the senescent phase, and pRb was dephosphorylated in all clones at 30 to 40 days of culture. Although both p53 and p21 expression decreased in all clones at 45 to 55 culture days, down-regulation of p16 and phosphorylation of pRb occurred only in clones that were able to bypass senescence (clones T5, T6, P3, P4, and P6; Fig. 2, right). Significant elongation of telomere was not observed even in cells that managed to evade senescence and continued to proliferate (data not shown). This suggests that, at least in long-term culture of human prostatic TAC, p16/pRb signals play important roles in senescence.

Subcloning of DU145 prostate cancer cells with various levels of junB. Confluent culture may damage cells, creating more avenues for growth selection. To obtain multiple malignant phenotypes, we cultured an androgen-independent prostate cancer cell line, DU145, and maintained them in stationary

Suppression of prostate cancer invasion by junB. Two DU145 clones, one with low junB expression (clone 2) and one with
Expression of junB in normal prostate epithelial cells and prostate cancer. Within the larger cohort of primary and metastatic prostate cancers examined immunohistochemically, we detected junB staining more frequently in normal epithelium (61.7 ± 3.45%) and low-grade lesions with Gleason scores (GS) of ≤6 (43.3 ± 4.44%). Cancers with a GS > 7 and metastatic lesions showed significantly lower levels of junB immunoreactivity (GS = 7, 38.6 ± 4.71% versus GS = 8, 11.6 ± 1.94%; metastatic tumors, 1.93 ± 0.25%). When present, staining was predominantly localized to the nuclei of normal epithelial and cancer cells. The correlation between junB immunoreactivity and lesion GS is illustrated in Fig. 6A and B.

Discussion

Our data indicate a previously unsuspected role for junB as an upstream regulator of p16/pRb signaling in the pathway leading to senescence of TAC in human prostate; further, we have found that silencing of junB can lead to reversal of cell cycle arrest even with activation of p16/pRb. Previous reports have delineated p53/p21- and p16-dependent pathways in reversible and irreversible senescence, respectively (19, 39–41). The p53/p21 pathway is executed in response to telomere erosion, but inactivation of p53 can reestablish cell proliferation. We found that p53 and p21 were up-regulated at entrance to senescence, after which protein levels decreased, but cells could not bypass the phase once started. Our data suggest that the p53/p21 pathway contributes to the start of senescence but is not required for sustaining the phase. On the other hand, active p16/pRb appears to be required not only for initiation but also for maintenance of senescence in TAC; once induced, silencing of p16 failed to reestablish cell growth (19). We detected increased levels of p16 and dephosphorylated pRb in
cells entering senescence, but, more significantly, p16/pRb signals were inactivated only in TAC that were able to bypass cell cycle arrest altogether. Cellular senescence dependent on p16 may be induced in response to telomere erosion and/or by another as yet unidentified stimulus. Maurelli et al. (42) report that, in primary human keratinocytes, senescence bypass is accomplished only when p16 is down-regulated in those cells endowed with a high proliferative potential. Moreover, elevated intracellular level of reactive oxygen species and protein kinase C activation has been found to maintain senescent human cell cycle arrest, resulting in blocking against immortalization, even after p16/pRb signals are inactivated (20). It would appear that p16 activation is a factor required for senescence initiation and maintenance and that, once p16/pRb is fully activated, senescence becomes irreversible regardless of subsequent p16/pRb status. JunB is a well-known member of the activating protein-1 family that negatively regulates cell proliferation either through direct activation of p16 or through inhibition of cyclin D1, which results in prolonged G1 phase (35, 36, 43). Moreover, junB can drive mouse fibroblasts to enter hydroxy-urea-dependent senescence (44). Thus, there is precedence for suspecting junB participation in initiating senescence but no previous data concerning the ability of junB to sustain senescence or permit bypass of cell cycle arrest. However, we now can state that, at least in prostate cancer cell lines, down-regulation of p16 can reverse senescence.

The percentage of TAC clones evading senescence in our study appeared to be related to their zone of origin in the prostate, the highest percent (6 of 52, 11.5%) originating from the PZ followed by TZ clones (5 of 82, 6.1%). Perhaps of some significance was the fact that no clones (0 of 65) isolated from the CZ of prostate samples escaped senescence. Analysis of a small subset of selected PZ, TZ, and CZ clones showed that p16 down-regulation and pRb phosphorylation was evident only in those clones that maintained proliferation and those clones that did so were TZ or PZ derived. The persistent growth arrest characteristic of clones obtained from the CZ was reversed by inactivation of junB by siRNA transfection. Distinct and specific genetic alterations in different chromosomes have been detected between PZ and TZ carcinomas (22), and basal cells

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**Fig. 4.** A to C, role of junB in prostate cancer invasion. A, DU145 cells were subcloned as described in Materials and Methods. Clones 1 to 4 were selected and expression of junB and p16 was examined by Western blotting (left). Cell viability was analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and expressed as ratios of mean ± SE absorbance to control (right). B, clones 2 and 4 were transfected with pEGFP and incubated for 16 h and then seeded atop the CAM of 11-day-old chicks and cultured for 5 days. C, left, microscopic findings and anti-GFP immunohistochemical staining of CAM cross-sections; C, right, invasion assessed by counting the number of invading cells per high-power field and quantifying front depth of CAM invasion by three or more cells. Mean ± SE of at least three experiments. Magnification, ×100 and ×400.
derived from the PZ and TZ exhibit different telomerase activity, hormonal receptors, apoptosis-related molecules, and cell cycle inhibitor proteins (23). Such molecular diversity may help explain why prostate cancer arises predominantly within the PZ and less often from the TZ or CZ and why prostate disease presents with such biological and clinical differences (45). It may be that the apparent inability of CZ TAC to escape senescence is a factor in the low incidence of carcinoma of CZ origin. In the preset research, all clones that bypassed senescence entered to the crisis, resulting in cell death showing apoptosis-like features, and none of them could evade from the phase. Now, we examine the key molecules other than junB involved in bypass of crisis, spontaneous transformation, and immortalization.

Our CAM assays also pointed to junB as a potent inhibitor of prostate cancer invasion as assessed by both in vivo and immunohistochemical analyses. Based on experiments in long-term TAC culture, we suspected that junB might function as a suppressor of prostate carcinogenesis and might affect other aspects of cancer, such as progression and malignant conversion. Using DU145 subclones with various constitutive levels of junB protein, we found that junB suppressed the invasive propensity of prostate cancer cells through inhibiting MMP2 induction. Moreover, in a large sampling of prostate tumor specimens, junB immunopositivity was significantly decreased specifically in those carcinomas scoring higher on the Gleason scale and was much lower still in metastatic cancers. To our knowledge, this is the first in vitro, in vivo, and immunohistochemical evaluation of junB function correlated to pathologic grading in human prostate cancer. There are a few studies examining the role of c-Jun, another member of the JNK family having antagonistic properties to junB, in prostate cancer. In one such study, c-Jun-specific immunopositivity was found to be stronger in the vast majority of prostate cancer cases than in prostatic hyperplasia, but no significant correlation to tumor histologic grade was detected (46). In addition, the phosphorylated form of c-Jun was highly expressed in androgen-independent prostate cancers, particularly in those with shorter patient survival times (47). It thus appears that c-Jun contributes to prostate cancer progression through its tumor-promoting effects, which are enhanced by decreased expression of junB. In other types of human malignancies, loss of junB expression is directly responsible for promoting granulocytic progenitors resembling chronic myelogenous leukemia; reexpression of junB fully reverts the hyperproliferative phenotype and induces terminal differentiation of granulocytes (48). There are several

![Fig. 5. Enhancement of CAM invasion by JunB gene silencing in prostate cancer cells.](image-url)

**Fig. 5.** Enhancement of CAM invasion by JunB gene silencing in prostate cancer cells. **A,** clones 2 and 4 were transfected with either 100 nmol/L junB siRNA or control RNA and incubated for 16 h and then seeded on the CAM of 11-day-old chicks and cultured for 3 days. **Left,** macroscopic findings and GFP immunohistochemistry of CAM cross-sections; **right,** cell grafts were collected after 3 days of incubation and junB protein and RNA expression was evaluated with Western blotting and reverse transcription-PCR, respectively. **B,** invasion was assessed by counting the number of invading cells per high-power field (**right**) and quantifying front depth of invasion by three or more cells (**left**). Mean ± SE of at least three experiments. Magnification, ×400.
studies focusing on the role of jun family members in MMP2 induction in various types of cells. Generally, mitogen-activated protein kinases, including c-Jun NH$_2$-terminal kinase, extracellular stress-regulated kinase, and the dependent transactivation of AP-1, lead to increased expression of MMP2 (49, 50); therefore, it is reasonable that junB as the antagonist to c-Jun might suppress tumor cell invasion through MMP2 inhibition as seen in the current study. We already surmised that p16 was a downstream target of junB, because our data supported this. We can reasonably conclude, therefore, that junB has two major downstream targets and signal effects, the first being induction of p16-associated cell cycle arrest (senescence) in nonmalignant cells and the second being inhibition of MMP in prostate cancer cells leading to a p16-independent suppression of invasion.

In summary, junB is an essential upstream regulator of p16/pRb and contributes not only to initiation but also to maintenance of cell senescence, which serves to block malignant transformation of prostatic TAC. Significantly, down-regulation of junB in cell senescence can revoke the cell cycle arrest and initiate proliferation. This finding modifies the conventional wisdom that cell senescence is irreversible after activation of p16/pRb. Cancer invasion in vivo is suppressed by elevated junB; moreover, nuclear expression of junB is inversely correlated with increasing pathologic grade in prostate cancer. More investigations are required to estimate the physiologic roles of progenitor and stem cells of human prostate; however, we believe that the present data targeting basal cell populations may provide important avenues for development of novel and efficacious strategies targeting junB in the prevention and treatment for human prostate cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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