Characterization of Phosphoglycerate Kinase-1 Expression of Stromal Cells Derived from Tumor Microenvironment in Prostate Cancer Progression

Jianhua Wang¹,4, Gigi Ying³, Jingchen Wang⁴, Younghun Jung⁴, Jian Lu¹, Jiang Zhu², Kenneth J. Pienta³, and Russell S. Taichman⁴

Abstract

Tumor and stromal interactions in the tumor microenvironment are critical for oncogenesis and cancer progression. Our understanding of the molecular events by which reactive stromal fibroblasts — myofibroblast or cancer-associated fibroblasts (CAF) — affect the growth and invasion of prostate cancer remains unclear. Laser capture microdissection and cDNA microarray analysis of CAFs in prostate tumors revealed strong up-regulation of phosphoglycerate kinase-1 (PGK1), an ATP-generating glycolytic enzyme that forms part of the glycolytic pathway and is directly involved in CXCL12-CXCR4 signaling. Normal fibroblasts overexpressing PGK1 resembled myofibroblasts in their expression of smooth muscle α-actin, vimentin, and high levels of CXCL12. These cells also displayed a higher proliferative index and the capability to contribute to prostate tumor cell invasion in vitro, possibly through expression of MMP-2 and MMP-3 and activation of the AKT and ERK pathways. Coimplantation of PGK1-overexpressing fibroblasts with prostate tumor cells promoted tumor cell growth in vivo. Collectively, these observations suggest that PGK1 helps support the interactions between cancer and its microenvironment. Cancer Res; 70(2); 471–80. ©2010 AACR.

Introduction

Prostate cancer (PCa) is a common neoplasm and is responsible for ~27,000 deaths yearly in American males (1). Neoplastic epithelial cells, which result mainly from the accumulation of somatic mutations (2), coexist in carcinomas with a highly complex stroma composed of a number of stromal cell types and extracellular matrix, both of which contribute to the complexity of the tumor microenvironment (3).

It is now well-documented that microenvironmental interactions in tumors are crucial in oncogenesis and cancer progression in a number of settings (4), including breast (5, 6), colon (7), lung (8), and prostate (9) cancers. Overall, increased numbers of fibroblasts are found in tumor stroma, which commonly have a myofibroblastic phenotype, and have been described as cancer-activated or cancer-associated fibroblasts (CAFs) as distinguished from normal fibroblasts (NSFs; 10). The presence of a reactive stroma has been shown to enhance capillary density and deposition of type I collagen and fibrin (10). In the prostate, activation of stromal fibroblasts has been shown to lead to cancer progression, and has a prognostic value (11–13).

Our previous studies have shown that stromal cell–derived factor 1 (SDF-1 or CXCL12) and its receptors (CXCR4 and RDC1/CXCR7) play important roles in PCa metastasis (14, 15). More recently, Orimo and colleagues showed that CAFs play a central role in promoting the growth of tumor cells through their ability to secrete CXCL12 (5, 6). In fact, Begley and colleagues, showed that NSFs derived from older men express more CXCL12 than NSF derived from younger men, suggesting that the stromal fibroblasts of older men may be “primed” to support cancer growth (16). However, whether stromal cells promote or inhibit prostate growth remains unsettled. For example, Degeorges and colleagues, developed stromal cell cultures from normal human prostate and benign prostatic hyperplasia composed of NSFs and myofibroblasts and studied their roles in modulating LNCaP, PC3, and DU145 PCa cell lines (17, 18). The benign prostatic hyperplasia cell line decreased LNCaP growth by 50% and DU145 growth by 40%, but did not inhibit the growth of PC3 cells (17, 18). Collectively, understanding of the molecular events for which the reactive stromal cells directly affect PCa progression, however, remains unclear.

Phosphoglycerate kinase-1 (PGK1) is an ATP-generating glycolytic enzyme that forms part of the glycolytic pathway and has been shown to be a downstream target of CXCL12/CXCR4 signaling in PCa cells (19). PGK1 is secreted...
extracellularly by tumors, where it acts as a disulfide reductase that can cleave plasminogen, generating the vascular inhibitor angiotatin (20, 21). Downregulation of PGK1 secretion by tumor cells at sites rich in CXCL12 provides a molecular mechanism to generate an angiogenic switch during metastasis (19).

Here, tissues obtained from a rapid autopsy program were used to identify PGK1 as a molecule expressed by CAFs, and was able to regulate PCa growth. Importantly, overexpression of PGK1 and CXCL12 in NSFs facilitated their conversion into cells bearing the CAI phenotype. These findings suggest that CXCL12 and PGK1 may serve as molecular switches that are able to drive cancer progression from both a stromal environment and cancer cell perspective.

Materials and Methods

Cell lines. The cell lines used in this study were derived from two human cancerous glands obtained from patients with PCa. The glands were dissociated, and the various cell types were then separated to obtain CAFs. NSFs were taken from a histologically noncancerous region of the prostate at least 2 cm away from the outer tumor margin. Their identities were then characterized by immunohistochemical stainings for CAF markers α-smooth muscle actin (α-SMA) and vimentin (25). The PC3 cells were cultured in growth medium, RPMI 1640 with 10% FCS, and antibiotics (Invitrogen, Corp.) at 37°C, 5% CO2.

Isolation of NSF and CAFs from normal and PCa tissues. All tissues were obtained from the radical prostatectomy series at the University of Michigan and from the Rapid Autopsy Program (22). Stromal nodules of benign prostatic hyperplasia and stroma adjacent to PCa foci (n = 2) were minced and enzymatically dissociated in mammary epithelial growth medium (Cambrex) supplemented with 2% bovine serum albumin (Fraction V; Fisher Scientific), 10 ng/mL of cholera toxin, 300 units/mL of collagenase (Invitrogen), and 100 units/mL of hyaluronidase (Calbiochem) at 37°C for 18 h. Later, tissue cells were recovered by differential centrifugation to separate the epithelial cells and fibroblasts (23). NSFs in the supernatant were pelleted and centrifuged at 800 rpm for 10 min followed by two washes with DMEM/F12 medium. The cell pellet was resuspended in DMEM/F12 medium supplemented with 5% fetal bovine serum (Invitrogen) and 5 μg/mL of insulin, and plated in 25 cm² tissue culture flasks. The culture was incubated and undisturbed for 2 to 3 d at 37°C, 5% CO2.

Laser capture microdissection and cDNA microarrays. Laser capture microdissection (LCM) was performed from frozen tissue sections with the SL Microtest device using CUT software (MMI). Approximately 5,000 to 10,000 cells were isolated from frozen prostate tissue specimens from separate cases of Gleason 3 + 3 tumors. Additional methodologies are provided in the Supplementary sections.

Construction of lentiviral vectors. For small interfering RNA knockdown of PGK1 or CXCL12, the iLentiv siRNA vector was purchased from Applied Biological Materials, Inc., and designed with convergent PolIII promoters. Five micrograms of each vector and 2.5 μg of each packaging vector were co-transfected into 293T cells using Lentifectin (Applied Biological Materials). Supernatants were collected at 36 to 48 h after purification and were used to infect NSFs and CAFs (NSFPGK1, NSFΔCXCL12 and CAFΔPGK1, CAFΔCXCL12). A PGK1 lentiviral vector was similarly prepared using a 1.33 kb human cDNA isolated by reverse transcription-PCR from total RNA extracted from PC3 cells. A 300-bp hCXCL12 cDNA was recovered from a pEB6-SDF-1 vector provided by Dr. Daisuke Uchida (Second Department of Oral and Maxillofacial Surgery, Tokushima University School of Dentistry, Tokushima, Japan; ref. 24) and was used to generate CXCL12 lentiviruses. NSFs or CAFs were infected with the PGK1 or CXCL12 expressing vector (NSFPGK1, CAFPGK1, NSFΔCXCL12, and CAFΔCXCL12) or empty control vectors (NSFControl or CAFControl). Control cells were transduced with lentigal vector or with the empty vectors. All transfected cells were selected in G418 (Invitrogen) and cells expressing high GFP levels were isolated by fluorescence-activated cell sorting.

ELISAs. Antibody sandwich ELISAs were used to evaluate PGK1 and CXCL12 expression in the NSFs or CAFs conditioned medium (CM; R&D Systems) as previously described (19).

Proliferation assays. After 24 h of serum withdrawal, PC3 cells were digested and washed three times in PBS and 1 × 10⁶ cells were incubated with a 1:1 mixture of CM derived from CAFΔgal, CAFΔPGK1, NSFΔCXCL12, NSFControl, NSFPGK1, or NSFΔCXCL12 and fresh growth medium in triplicate 96-well flat-bottomed tissue culture plates in a total of 0.1 mL. Additional methodologies are provided in the Supplementary sections.

Invasion assays. Cell invasion was examined using a reconstituted extracellular matrix membrane (BD Biosciences). PC3 cells were placed in the upper chamber (1 × 10⁵ cells/well) in serum-free medium, and CM derived from altered expression of CXCL12 and PGK1 in NSFs, CAFs, and the respective controls were added to the bottom chambers. Additional methodologies are provided in the Supplementary sections.

Western blot analyses. PCa, NSFs, or CAFs were cultured to confluence, washed, and then serum-starved in RPMI with 0.1% bovine serum albumin for 24 h. The cells were lysed in ice-cold radioimmunoprecipitation assay buffer. Cell lysates were clarified by centrifugation at 14,000 rpm for 10 min and protein concentrations were determined (Bio-Rad Laboratories). Additional methodologies are provided in the Supplementary sections.

In vivo assay for the effects of NSFs on tumor development. To determine whether PGK1 activated fibroblast-supported PCa progression in vivo, 1 × 10⁶ PC3ΔΔi cells alone or mixed 1:1 with either CAFControl, NSFControl, NSFPGK1, or NSFΔCXCL12 were implanted s.c. into the backs of severe combined immunodeficient mice (CB.17.SCID; Taconic) under isoflurane. After 4 wk, bioluminescence imaging was used to monitor the tumor growth. The mice were injected i.p. with luciferin (100 μL at 40 mg/mL in PBS) before imaging. This dose and route of administration has been shown to be optimal for rodent studies within 10 to 20 min after luciferin administration.
injection (14). Mice were anesthetized with 1.5% isoflurane/air, and the Xenogen IVIS cryogenically cooled imaging system was used as described (14). Selected mice were imaged weekly after tumor injection to monitor tumor development. Bioluminescence generated by the luciferin/luciferase reaction served as a locator for cancer growth and was used for quantification using the LivingImage software on a red (high intensity/cell number) to blue (low intensity/cell number) visual scale. A digital grayscale animal image was acquired followed by acquisition and overlay of a pseudocolor image representing the spatial distribution of detected photon counts emerging from active luciferase within the animal. Signal intensity was quantified as the sum of all detected photons within the region of interest during a 1-min luminescent integration time. All animal procedures were performed in compliance with institutional ethical requirements and were approved by the University of Michigan Committee for the Use and Care of Animals.

**Immunohistochemistry.** For immunostaining with α-SMA, vimentin, and fibroblast activation protein (FAP; Figure 1. Staining of PCa tissue microarrays for α-SMA shows a CAF transition. A, tissue microarray was constructed from 30 patients to represent benign, primary, and PCa metastasis sites. Three cores (0.6 mm in diameter) were taken from each representative tissue block. The tissue microarray was immunostained for α-SMA. Few cells in the stroma of normal prostates stained positive for α-SMA (A1, arrows). In primary PCa, however, virtually all of the stromal cells stained positive for α-SMA (A2, arrows). B, flow chart for experimental design showing LCM and two rounds of T7 amplification.

<table>
<thead>
<tr>
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<th>Overexpression rank</th>
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<td>PDLIM3</td>
<td>5.2695</td>
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<td>THBS1</td>
<td>4.4057</td>
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<td>Thrombospondin—cell adhesion, inhibits angiogenesis</td>
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<td>ACTC</td>
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<td>α-SMA—epithelial-to-mesenchymal transition</td>
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<td>49</td>
<td>Vimentin—expression marker of epithelial-to-mesenchymal transition</td>
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Abcam), tissue sections were blocked with Sniper for 5 min, and incubated overnight at 4°C with 2.8 μg/mL rabbit anti-human α-SMA, vimentin, and FAP diluted in PBS. The sections were incubated with the appropriate secondary antibodies for 30 min, followed by processing with a Lincoln Label 41 Detection System (Biocare Medical). Additional methodologies are provided in the Supplementary sections.

Figure 2. Reciprocal expression of CXCL12 and PGK1 by tumor stromal cells. A, expression of α-SMA is higher in CAFs than NSFs (green). Representative staining of vimentin and FAP (brown) in cultures of NSFs and CAFs. Hematoxylin was used as a counterstain. Original magnification, ×40 (bar, 50 μm). B, CXCL12 and PGK1 levels in NSFs and CAFs CM. CXCL12 and PGK1 levels (1 × 10⁶ cells/well) were evaluated by ELISA at 24 and 48 h. Columns, mean for triplicate determinations normalized against total protein; bars, SD. *, P < 0.05, significant difference from respective controls (ANOVA; n = 6 in groups). C and D, ELISA assays for CXCL12 and PGK1 levels in CM derived from NSFs and CAFs which overexpress or have reduced levels of CXCL12 and PGK1 (denoted as NSFCXCL12, NSFPGK1/NSFControl, CAFCXCL12, CAFPGK1/CAFControl, CAFsiCXCL12, or CAFsiPGK1/CAFβ-gal). A β-gal sequence was incorporated into the small interfering RNA as a vector control. Columns, mean for triplicate determinations normalized against total protein; bars, SD. *, P < 0.05, significant difference from respective controls (ANOVA; n = 6 in groups).
Statistical analyses. Numerical data are expressed as mean ± SD. Statistical differences between the means for the different groups were evaluated with Instat 4.0 (GraphPad software) using one-way ANOVA, with the level of significance set at \( P < 0.05 \).

Results

**PGK1 expression is upregulated in CAF versus NSF derived from the prostate peripheral zone by LCM and cDNA microarray assay.** To explore the role of tumor stroma in PCa development, immunohistochemical stainings for \( \alpha \)-SMA in PCa tissues was performed and compared with normal prostate. We found that a few cells in the stroma from the normal prostate tissues stained positively for \( \alpha \)-SMA (Fig. 1A1, arrows). In primary PCa, however, virtually all stromal cells expressed \( \alpha \)-SMA (Fig. 1A2, arrows), as has been reported elsewhere (23, 25).

\( \alpha \)-SMA was next used to stain serial tissue sections and CAFs were isolated by LCM. cDNA microarrays were performed using RNA recovered from CAFs compared with genes expressed in normal NSFs (Fig. 1B). SAM analysis and average linkage hierarchical clustering were performed and the output was visualized with TreeView software (26) to compare the expression profiles of CAFs with NSFs. The expression of select genes upregulated in stroma during the conversion to cancer is presented in Table 1. Among those genes whose expression was most highly upregulated in CAFs versus NSFs derived from the prostate peripheral zone was PGK1.
A facilitating relationship between CXCL12 and PGK1 exists in tumor stromal cells. In previous studies, the relationship between CXCL12 and CXCR4 signaling and the expression of PGK1 in PCa was explored (19). Here, to determine how CXCL12 and PGK1 expressed by CAFs regulate tumor progression, stromal cells were first characterized for the expression of the CAF markers α-SMA, FAP, and vimentin. As shown in Fig. 2A, expression of α-SMA, vimentin, and FAP were higher in CAFs than NSFs. Next, the levels of CXCL12 and PGK1 in the CM of CAFs and NSFs were determined. Higher levels of CXCL12 were secreted by the CAFs relative to the NSFs (Fig. 2B). Likewise, the levels of PGK1 secreted from CAFs were higher than levels produced by NSFs (Table 1).

To further explore the role of CAF-secreted CXCL12 and PGK1 in PCa progression, lentiviral vectors were used to over-express or reduce CXCL12 and PGK1 expression in NSFs and CAFs. After clonal selection, individual clones were pooled and assayed by ELISA for CXCL12 and PGK1 expression (Fig. 2D). Interestingly, overexpression of CXCL12 in NSFs and CAFs enhanced the secretion of PGK1 compared with controls. In contrast, reduced CXCL12 expression inhibited PGK1 expression by CAFs. These data suggest that expression of CXCL12 in stromal cells directly regulates PGK1 levels (Fig. 2D).

To determine if PGK1 alters CXCL12 levels, overexpression and knockdown of PGK1 in NSFs and CAFs were performed. Overexpression of PGK1 increased the expression of CXCL12 compared with controls. In contrast, reducing PGK1 expression decreased the expression of CXCL12 levels by CAFs (Fig. 2D). Together, these data further suggest a direct and facilitating relationship between CXCL12 and PGK1 levels in the tumor stroma (Fig. 2D).

Previously, we found that expression of PGK1 in tumor cells was inversely correlated with CXCR4 level (19). To determine the relationship between the expression of PGK1 and CXCR4 in tumor stromal cells, quantitative reverse transcription-PCR and Western blot assays were performed. Supplementary Fig. S1 shows that reduced CXCR4 levels were observed in NSFs that overexpressed PGK1 and CXCL12, consistent with the results shown in CAFs. Similar results were also observed for CXCR7 levels, a second CXCL12 cognate receptor. These data suggest that expression of PGK1 in tumor stromal cells was also inversely regulated by CXCL12 receptors.

CXCL12/PGK1 expression in NSFs stimulates PCa proliferation and invasion. Next, we evaluated whether the expression of CXCL12 or PGK1 in NSFs and CAFs affects their phenotype. As shown in Supplementary Fig. S2, overexpression of CXCL12 and PGK1 in NSFs induced morphologic changes reminiscent of the CAF cell phenotype. In addition, overexpression of CXCL12 or PGK1 in NSFs resulted in a higher proliferative rate, in which reduced expression of CXCL12 or PGK1 produced a reduction in Ki-67 levels (Fig. 3A).

To determine if the expression of CXCL12 and PGK1 by NSFs and CAFs was able to alter PCa growth, CM was collected and was cocultivated with PC3 cells. PC3 growth rates were enhanced in the presence of CM derived from NSFPGK1 and NSF CXCL12 at day 5, compared with the CM derived from NSFControl cells. In keeping with the previous results, PC3 growth rates were significantly attenuated in the presence of CM derived from CAF CXCL12 and CAF PGK1 cells, compared with the CM controls (Fig. 3B).

Given that alterations in the expression of PGK1 and CXCL12 by stromal constituents were able to alter the proliferation of PCa cells, it was next determined if similar effects could be observed on the invasive activity of PCa. Using reconstituted extracellular matrices in porous culture chambers, PC3 cells were placed in the upper chamber (1 × 10^5 cells/well) in serum-free medium, and CM derived from the panel of NSFs and CAFs expressing different levels of CXCL12, and PGK1 was added to the bottom chambers. Invasion into the matrix was determined after 48 hours of culture. CM containing high levels of CXCL12 expressed by...
both NSFs and CAFs supported the invasion of PC3 as compared with the respective controls, consistent with previous reports (refs. 16, 27; Fig. 3C). CM derived from cells engineered to overexpress PGK1 increased the invasiveness of PC3 (Fig. 3C), whereas decreasing PGK1 and CXCL12 expression reduced the invasive abilities of PC3 compared with controls (Fig. 3C). Moreover, adding a CXCL12-neutralizing antibody in CM from the NSFPGK1 cells decreased the effects of PC3 proliferation relative to an IgG antibody control. Similar results were also observed in PC3 cells pretreated with CXCR4 inhibitors before the addition of CM from the NSFPGK1 cells. These data further suggest that the CXCL12 secreted from NSFPGK1 cells activates CXCR12 receptors in PCa cells, which is associated with proliferation and invasion (Supplementary Fig. S3).

Expression of PGK1 and CXCL12 by NSF contributes to expression of CAF phenotype. Given the effects of overexpression of PGK1 and CXCL12 in NSFs and CAFs on tumor proliferation, we hypothesized that expression of these genes contributes to the expression of a CAF phenotype. Figure 4A and B show that the expression of α-SMA and vimentin was higher in CAFs than NSFs under basal conditions. Overexpression of PGK1 and CXCL12 in NSFs induced higher expression of vimentin compared with controls. Similarly, overexpression of PGK1 in NSFs increased a higher level of α-SMA but not in NSFs overexpressing CXCL12. These data support the notion that expression of PGK1 may induce the differentiation of NSFs into a stage that resembles CAFs.

CXCL12 and PGK1 expression in NSFs promotes PCa growth in vivo. To determine whether overexpression of PGK1 in NSFs supports PCa progression in vivo, an equal number of PC3luc cells alone or mixed with either CAFcontrol, NSFPGK1, or NSF CXCL12 cells were implanted s.c. into the backs of nonobese diabetic/severe combined immunodeficient mice.

Figure 5. Overexpression of CXCL12 or PGK1 in NSFs facilitates PCa growth in vivo. A, PC3luc cells were implanted alone or mixed with NSF CXCL12, NSFPGK1, NSFcontrol, or CAFcontrol cells at a ratio of 1:1 cells into the backs of nonobese diabetic/severe combined immunodeficient mice. At 4 wk, imaging was performed with a Xenogen IVIS imaging system. Luciferase signals from the representative mice are shown. B, quantification of tumor burdens identified by bioluminescence imaging. Increasing tumor size was observed after coinjection of NSF CXCL12 or NSFPGK1 compared with NSFcontrol cells. Coimplantation of CAFcontrol cells served as a positive control. *, P < 0.05, significant difference from controls. #, P < 0.05, significant difference from presence of CAFcontrol or PC3 alone. C, immunofluorescence staining for CAF markers α-SMA and vimentin in tumor stroma from NSFcontrol, NSF CXCL12, and NSFPGK1 mixed with PC3luc. Nuclei were identified by 4′,6-diamidino-2-phenylindole. All images were captured on Zeiss LSM 510 meta confocal laser scanning microscope. Original magnification, ×60; bars, 30 μm. D, quantitative evaluation of the expression of PGK1 in human PCa. Left, immunostaining intensity was scored as either absent (1), weak (2), moderate (3), or strong (4). The mean expression scores multiplied by percentage of positive cells in the field (Quick’s combined score system) for all benign, prostatic intraepithelial neoplasia (PIN), and localized cancer. Right, ELISA analysis of PGK1 level in serum of normal controls and patients with PCa. Points, mean for triplicate determinations normalized against total protein; bars, SD. *, P < 0.05, significant difference from respective controls (ANOVA; n = 3 in groups).
A similar pattern of staining for vimentin was shown in tumors derived from the PCa cells treated with CM derived from NSFPGK1 or NSFCXCL12, but not with NSFControl (Fig. 5D). In addition, PGK1 serum levels were higher in patients with PCa compared with age-matched controls (Fig. 5D). These findings suggest that PGK1 may indeed play a role in PCa progression (20, 28).

### Molecular mechanisms of PCa progression modulated by PGK1

Based on these *in vitro* and *in vivo* findings, we further explored the molecular mechanisms by which PGK1 induces CAF formation, and subsequently, PCa progression. The differences in mRNA levels of select genes were explored in NSFControl, NSFPGK1, or NSFCXCL12 cells using microarray assays. Supplementary Table S1 shows that the top seven pathways enriched in common genes induced by overexpression of CXCL12 and PGK1 in NSFs were involved in resistance to apoptosis (e.g., CADM1, MAPK10, and HIPK3), promoted cell proliferation (e.g., CXCL12, ADAMTS1, and E2F1), were involved in metastasis [e.g., matrix metalloproteinases (MMP-2, MMP-3, MMP-9, MMP-14), MTA1, and CD44], activated MAPK and Wnt pathways (e.g., MAP2K7, P2PP2CA, and DKKI), or associated with cell-cell adhesion, including VIM, SMARC1, CAV2, and others. These results were partially validated by focusing on α-SMA, MMP-2 and MMP-3 using Western blots. As shown in Fig. 6A, expression of MMP-2 and MMP-3 levels were higher in NSFs overexpressing PGK1 and CXCL12 compared with controls. Expression of α-SMA was higher in NSFPGK1 or NSFCXCL12 cells relative to NSFControl cells (Fig. 6A). Yet, overexpression of PGK1 in CAF did not greatly alter the expressions of α-SMA, MMP-2, and MMP-3.

Finally, the pathways activated in PCa cells themselves when they interact with stromal cells were explored. PCa cells are cultured with CM derived from NSFs overexpressing PGK1 or CXCL12. Here, activation of the AKT pathway in PC3 cells was observed at 10 and 30 minutes compared with CM derived from NSFControl (Fig. 6B). Similarly, the ERK pathway was also activated in PCa when the cells were treated with CM derived from NSFPGK1 or NSF CXCL12 cells (Fig. 6B).

### Discussion

Tumors arise from cells that have sustained genetic mutations resulting in deregulation of normal growth control mechanisms (3). Research focused on the origins of cancer has identified that genetic mutations within tumor cells have resulted in the concept that neoplastic progression is a cell-autonomous process. However, it has become increasingly apparent that the microenvironment influences many steps in tumor progression (29). Not only do the cancer cells interact with each other, they are also influenced by the extracellular matrix and other microenvironmental cells including fibroblasts, endothelial cells, and inflammatory cells, etc. (9, 29). Recent work has shown that alterations in...
the stromal compartment often accompany, and may even precede, the malignant conversion of epithelial cells. The conversion of normal tissue-resident fibroblasts into CAFs seems to be a critical step in neoplastic progression (10, 30).

Here, LCM was performed in conjunction with cDNA microarray analysis of noncancerous prostate tissues, primary PCa, as well as PCa metastases to differentiate the contributions of cancer epithelial cells versus supporting stromal cells (Fig. 1A). Our data showed significant alterations in the expression of genes associated with the transition of NSFs to CAFs. In conjunction with the transition of the stroma to a CAF phenotype, there was a significant shift in the expression of a multitude of genes associated with angiogenesis inhibition, including PGK1, and ADAMTS1, which by itself has been shown to stimulate PCa growth (ref. 27; Table 1).

Additional studies exploring the mechanisms by which CAFs induce tumor progression and stromal-epithelial interactions were modeled in vitro and in vivo. We found that expression of PGK1 in NSFs supported the expression of CAF differentiation including the expression of α-SMA, vimentin, FAP, and CXCL12. Furthermore, overexpression of CXCL12 or PGK1 in NSFs enhanced proliferation and modulated PCa invasion in vitro. These data suggest that although the expression of PGK1 has a role as an inhibitor of angiogenesis (19, 21), it also supports CAF-like features and regulates CXCL12 secretion. Together, these features may create a microenvironment that supports tumor growth and invasion.

We have also shown that expression of PGK1 and CXCL12 in NSFs alters a number of pathways which are associated with tumor progression, including a number of the MMPs (e.g., MMP-2, MMP-3, MMP-9, and MMP-14). Moreover, AKT and ERK pathways were activated in PC3 cells when cultured with CM from NSFs overexpressing PGK1. MMPs have been implicated in the promotion of tumor growth, angiogenesis, invasion, and metastasis, in which elevated MMP levels were associated with poor prognosis (31–33). In this study, the observation that PGK1 induced MMP-2 and MMP-3 expression in NSFs may provide the activation of critical molecules required to promote epithelial-to-mesenchymal transition, including cleavage of the extracellular domain of E-cadherin (34). Intriguingly, activation of AKT and ERK pathways in PCa by NSFs overexpressing PGK1 indicated that stromal cells support tumor growth and invasion.

One of the interesting aspects of this work was found when coinplantation studies were performed. Here, PC3luc cells alone or mixed 1:1 with either CAFcontrol, NSFcontrol, NSFPGK1, or NSFCXCL12 were injected into the backs of severe combined immunodeficient mice. The data showed that compared with the CAFcontrol cells, NSFPGK1 or NSFCXCL12 cells did not support robust in vivo tumor growth. One possibility is that PGK1-activated fibroblasts may not have completed the transition to CAF. However, in the presence of the fibroblast group, NSFPGK1 or NSFCXCL12 facilitated tumor growth to a higher extent than NSFcontrol cells (Fig. 5A and B). Moreover, it was interesting to note that NSFs inhibited PCa growth when the PCa cells were grown without NSFs (Fig. 5A and B). These data suggest that early NSFs may be protective, but as they move towards a CAF phenotype, the expression of PGK1 or SDF-1 in tumor stroma may drive tumor development.

Previously, we reported that PGK1 was a critical target of the CXCL12/CXCR4 chemokine axis, and suggested that a “Warburg effect” is likely to be a fundamental property of cancer cells and was known to correlate with enhanced tumor progression (19). Here, our observations indicate that PGK1 also plays a central role in inducing fibroblasts towards the CAF phenotype in the tumor microenvironment. One possible role of PGK1 in stroma may have the ability to increase metabolism and cell cycling, thus eliciting a CAF phenotype. This suggests that a “Warburg effect” may also be associated with tumor stromal cells; however, further studies are needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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