A Potential Oncogenic Activity of Platelet-Derived Growth Factor D in Prostate Cancer Progression

Carolyn V. Ustach, Marcus E. Taube, Newton J. Hurst, Jr., Sunita Bhagat, R. Daniel Bonfil, Michael L. Cher, Lucia Schuger, and Hyeong-Reh Cho Kim

Departments of 1 Pathology and 2 Urology, Barbara Ann Karmanos Cancer Institute, Wayne State University, School of Medicine, Detroit, Michigan

ABSTRACT

The platelet-derived growth factor (PDGF) proteins are potent stimulators of cell proliferation/transformation and play a major role in cell-cell communication. For over two decades, PDGFs were thought to exist as three dimeric polypeptides (the homodimers AA and BB and the heterodimer AB). Recently, however, the PDGF C and D chains were discovered in a BLAST search of the expressed sequence tag databases. The PDGF CC and DD dimers have a unique two-domain structure with an N-terminal CUB (compliment subcomponents C1r/C1s, Uegf, and Bmp1) domain and a COOH-terminal PDGF/vascular endothelial growth factor domain. Whereas secreted PDGF AA, BB, and AB readily activate their cell surface receptors, it was suggested that extracellular proteolytic removal of the CUB domain is required for the PDGF/vascular endothelial growth factor domain of PDGF CC and DD to activate PDGF receptors. In the present study, we examined the processing of latent PDGF D into its active form and the effects of PDGF D expression on prostate cancer progression. We show that LNCaP cells auto-activate latent PDGF DD into the active PDGF domain, which can induce phosphorylation of the β-PDGFR receptor and stimulates LNCaP cell proliferation in an autocrine manner. Additionally, LNCaP-PDGF D-conditioned medium induces migration of the prostate fibroblast cell line 1532-FTX, indicating LNCaP-conditioned medium can activate prostate carcinoma cell interaction with surrounding stromal cells. These demonstrate a potential oncogenic activity of PDGF DD in the development and/or progression of prostate cancer.

INTRODUCTION

Primary prostate carcinomas typically grow slowly and often do not progress to clinically relevant disease (1). The main cause for mortality from prostate cancer is metastasis, a complex process involving cancer cell invasion through stromal components and colonization at the distant organs (2, 3). Although it has become clear that paracrine signaling between the tumor cells and the surrounding mesenchymal cells plays a critical role for tumor progression, the molecular mechanisms of these interactions are much less understood.

The platelet-derived growth factor (PDGF) protein family is a potent stimulator of cell proliferation, chemotaxis, and transformation and is known to play a major role in cell-cell communication for normal development and also during pathogenesis (reviewed in Refs. 4 and 5). PDGF isoforms exert their biological functions through activation of two structurally related cell surface receptor tyrosine kinases [α-PDGFR receptor (PDGFR) and β-PDGFR (4, 6)]. Immunohistochemical analysis showed that ~80% of prostate tumor tissues express PDGFRs at both primary and metastasized sites (7). Interestingly, β-PDGFR staining is more prominent in endothelial cells when they are exposed to prostate tumor cells that express PDGF (8), suggesting significance of PDGF signaling for prostate cancer progression in a paracrine manner. Consistently, PDGFR inhibitor ST1571 (Gleevec) in combination with paclitaxel was shown to substantially reduce prostate cancer bone metastasis in a mouse model (8).

For more than two decades, PDGFs were thought to exist in the form of three dimeric polypeptides (the homodimers PDGF AA and BB and the heterodimer PDGF AB). The PDGF A chain can activate α-PDGFR only, whereas the PDGF B chain activates both α- and β-PDGFR (4, 6). Recently, however, the PDGF C and D chains were discovered in a BLAST search of the expressed sequence tag databases at the National Center for Biotechnology Information (9–11). The PDGF C and D chains have a unique two-domain structure with an N-terminal CUB (compliment subcomponents C1r/C1s, Uegf, and Bmp1) domain and a COOH-terminal PDGF/vascular endothelial growth factor domain. Whereas secreted PDGF AA, BB, and AB can readily activate their cell surface receptors, it was suggested that extracellular proteolytic removal of the CUB domain is required for the growth factor domain of PDGF CC and DD dimers to preferentially activate αα-PDGFR and ββ-PDGFR, respectively (9–11).

Whereas the PDGF A- and B-mediated signal transduction pathways leading to specific cellular processes have been extensively studied, little is known about the functions of the new family members, PDGF C and D. Increased expression of PDGF D has been found in ovarian, lung, renal, and glioma-derived cell lines as well as in clinical tumor samples, suggesting a correlation between increased PDGF D expression and human cancer (12, 13). Because β-PDGFR is thought to be critical for prostate cancer progression, and PDGF D is a specific ligand for β-PDGFR, we examined the effects of PDGF D expression in prostate cancer cell growth and its interactions with stromal cells. Whereas the previous studies suggested that the processing of latent PDGF DD into its active form requires proteases in serum (9, 10), we show in this study that human prostate carcinoma LNCaP processes latent PDGF DD into the active form under serum-free conditions, resulting in promotion of LNCaP growth. Additionally, PDGF DD produced by LNCaP cells induces cell motility of the prostate fibroblast cell line TFX-1532, indicating paracrine signaling of PDGF DD for the interactions between carcinoma and mesenchymal cells. We also show that PDGF DD expression greatly enhances prostate carcinoma cell interaction with the surrounding stromal layers in a severe combined immunodeficient (SCID) mouse model, demonstrating a potential oncogenic activity of PDGF D in human prostate cancer progression.

MATERIALS AND METHODS

Production of Recombinant PDGF D (rPDGF D) Protein. A two-step reverse transcription-PCR approach was taken to clone PDGF D into a vaccinia expression vector. Total RNA isolated from the prostate cell line DU145 using Trizol reagent was used in a cDNA synthesis reaction using SuperScript RTII (Invitrogen, Carlsbad, CA). The resultant cDNA was used in a first PCR reaction that yielded a 2107-bp product containing the 1113-bp open reading frame of PDGF D (forward, 5′-gga-gagcactgtctgta-3′; reverse, 5′-ttcctagctaggat-3′). This PCR product was gel purified and used as a template in a second PCR reaction designed to add the restriction enzyme sites AflIII and
BclI at the 5' and 3' ends, respectively, of the PDGF-D open reading frame, as well as the addition of a 6×His epitope tag at the carboxyl end of the PDGF-D protein (forward, 5'-ccagttgcgacgccgctcatctt-3' and reverse, 5'-ccagtgcgtcaaataggtgatcggcgctgtg-3'). The resultant 1150-bp product was digested with AflII and BclI, and inserted into the Neo/BamHI site of the vaccinia virus expression vector pTF7-ECM1 (a kind gift from Dr. R. Fridman, Wayne State University). pTF7-ECM1 contains the T7 promoter upstream of the Neo/BamHI cloning site; therefore, transcription of the inserted gene requires the presence of the T7 polymerase, supplied by infecting the cells with recombinant vaccinia virus. Fidelity of the in-frame sequence encoding the PDGF-D:His fusion protein was confirmed by DNA sequencing (Eli Lilly Pharmaceuticals, Inc., San Francisco, CA). This plasmid referred to as pTF7-PDGF-D:His and was used to produce recombinant full-length PDGF-D following established vaccinia virus protocols (14). In short, BS-C-1 (Green monkey kidney) cells were first infected with the recombinant vaccinia virus vTF7-3, which expresses the T7 RNA polymerase. After 30 min of infection, the cells were washed with PBS and then transfected with the plasmid pTF7-PDGF-D:His, using Effectene reagent (Qiagen). Expression of PDGF-D inserted into the pTF7 plasmid is reliant on infection of the cells by vTF7-3, which expresses the T7 RNA polymerase gene. Cell-host machinery then transcribes the gene of interest. Forty-eight h after co-infection/transfection with vaccinia virus and PDGF-D, the serum-free conditioned medium (CM) was collected and cleared of cellular debris by a 5-min centrifugation at 2000 × g. The resultant CM containing recombinant full-length PDGF-D was used in subsequent PDGF-D processing experiments.

Construction of a Mammalian PDGF-D Expression Vector. To insert PDGF-D into the mammalian expression vector pcDNA3, pTF7-PDGF-D:His (described in the preceding paragraph) was used as the template in a PCR reaction with the primers (forward, 5'-ctgtcagttgcgacgccgctcatctt-3' and reverse, 5'-ccagtgcgtcagctaggtgatcggcgctgtg-3') to add an AflII site at the 5' end and to delete the BclI site at the 3' end of the PDGF-D:His open reading frame. The subsequent 1150-bp PCR product was then digested with AflII and BclI and inserted into the AflII/BamHI site of the mammalian expression vector pcDNA3.1(+)(Invitrogen). This plasmid referred to as pcDNA3-PDGF-D:His and was used to create the stably transfected cell line LNCaP-PDGF-D.

Cell Culture. Human prostate carcinoma LNCaP cells and resultant transfected lines were cultured in a humidified 5% CO2 incubator with RPMI 1640 with 10% fetal bovine serum, pooled together, and are referred to as LNCaP-PDGF-D and LNCaP-neo, respectively. Overexpression of PDGF-D was confirmed by reverse transcription-PCR as follows: total RNA was extracted from the cells using Trizol reagent (Invitrogen). One µg of total RNA from each cell line was used to synthesize cDNA using Superscript RTII (Invitrogen). Resultant cDNA was used as template in a PCR reaction, using Taq polymerase (Promega, Valencia, CA) and the following primers: forward, 5'-gaaacagctttcgccgctgaacag-3' and reverse, 5'-ccggtcgagatcggcgctgtg-3'. These primers amplify a 192-bp product that represents part of the CUB coding region of the PDGF-D protein. This primer pair does not allow for discrimination between endogenously and exogenously expressed PDGF-D. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was used as a positive control with the primer pair 5'-ttaccttacgccgctgag-3' (forward) and 5'-gcggctgagacgccctga-3' (reverse).

Custom Antibody (Ab) Raised against the Growth Domain of PDGF-D. An Ab was raised against PDGF-D in rabbits using synthetic peptides (N'-RKSKVDDLRLNDADKRYSC' representing a portion of the growth factor domain of PDGF-D (amino acids 254–272). The resultant Abs were affinity purified (Zymed Biomedical, South San Francisco, CA) and were referred to as anti-PDGF-D/DIG Ab.

PDGF-D-Mediated Paracrine Activation of β-PDGFR and Extracellular Signal-Regulated Protein Kinases (ERKs). LNCaP-PDGF-D and LNCaP-neo cells were cultured in serum-free medium for 48 h. CM was collected and centrifuged at 2000 rpm for 5 min to remove cell debris. Serum-starved NIH3T3 cells were treated with CM collected from LNCaP-PDGF-D or LNCaP-neo cells for 10 or 60 min and lysed in immunoprecipitation assay lysis buffer [0.5% sodium deoxycholate, 1% NP40, 50 mM Tris (pH 7.6), 2 mM EGTA, 2 mM EDTA, 150 mM NaCl, 2 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin, and 10 µg/ml aprotinin]. The lysates were centrifuged for 20 min at 12,000 × g to remove debris, and protein concentrations were determined using the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL). Lysate (250 µg) was used for immunoprecipitation with an anti-β-PDGFR Ab [Santa Cruz Biotechnology (Santa Cruz, CA) or Oncogene Research Products (San Diego, CA)] and protein G-agarose beads (Pierce Biotechnology). Immunoprecipitates were washed five times with immunoprecipitation assay buffer and resolved by reducing SDS-PAGE. Tyrosine-phosphorylated β-PDGFR was detected by immunoblot using an anti-phosphotyrosine Ab (Upstate Biotechnology, Lake Placid, NY). Total levels of immunoprecipitated β-PDGFR were detected using the same Ab used for immunoprecipitation. To determine the total and activated levels of ERK1/2, 50 µg of lysate from each sample were resolved by reducing SDS-PAGE, and immunoblots were performed using anti-ERK1/2 or anti-ERK2 Abs according to the manufacturer’s instructions (Cell Signaling Technologies, Beverly, MA).
RESULTS

Human Prostate Carcinoma LNCaP Processes PDGF D into Active Growth Factor Domains. To examine the effects of PDGF D expression on human prostate carcinoma cell growth and tumorigenicity, LNCaP-PDGF D and LNCaP-neo cell lines were established as described in “Materials and Methods.” PDGF D overexpression in LNCaP-PDGF D was confirmed by reverse transcription-PCR analysis (Fig. 1A) as well as immunoblot analysis using anti-PDGF D/GD Ab (Fig. 1B). It was shown previously that when a PDGF D expression vector was transfected into human embryonic kidney (HEK 293) cells, only the full-length PDGF D protein was secreted from HEK 293 cells under serum-free conditions; HEK 293-secreted pro-PDGF D was found to be processed into an active growth factor dimer by unknown protease(s) in serum (10). Surprisingly, however, immunoblot analysis of secreted proteins collected from LNCaP-PDGF D cells in the absence of serum revealed that both the pro-form (M, 50,000) and the processed forms (M, 20,000 and 15,000), with sizes similar to what were reported to be growth factor monomers (9, 10), were detected under reducing condition using anti-PDGF D/GD Ab (Fig. 1B).

The above results suggest that the pro-PDGF D protein may be processed into the growth factor domain by protease(s) expressed in human prostate carcinoma LNCaP cells independent of serum. To examine PDGF D processing by proteinases produced by LNCaP cells, rPDGF D protein was produced using a vaccinia mammalian cell expression system as described in “Materials and Methods.” When BS-C-1 cells were cultured in serum-free medium after co-infection/transfection with vaccinia virus and pTF7-7-PDGF D:HIS (see “Materials and Methods”), only pro-PDGF D protein was detected, migrating as a dimer with a M of ~90,000 under nonreducing conditions and as a monomer with a M of ~50,000 under reducing conditions (Fig. 2A, left panel). In agreement with previous reports (9, 10), the pro-PDGF D protein was processed into the growth domain in the presence of serum, migrating as a dimer with M, ~35,000 and as monomer species with M, 20,000 and 15,000 (Fig. 2A, right panel). Using rPDGF D, we examined whether pro-PDGF D can be processed by secreted proteinase(s) or by cell surface/pericellular proteinase(s) produced from LNCaP cells. Whereas CM collected from LNCaP cells contained little proteolytic enzyme capable of processing rPDGF D (Fig. 2B, right panel), incubation of rPDGF D with LNCaP-neo cells resulted in cleavage of rPDGF D, suggesting that cell surface or pericellular proteinase(s) may be responsible for processing pro-PDGF D into the growth factor domain (Fig. 2B, left panel). It was noticed that rPDGF D was processed into several species with M, ~25,000 by proteinase(s) produced by LNCaP-neo cells, whereas two major monomer species with M, 20,000 and 15,000 are usually detected in CM collected from LNCaP-PDGF D cells (Fig. 1B). Next, we examined whether rPDGF D is more effectively processed into M, 20,000 and 15,000 monomers in the presence of LNCaP-PDGF D.
cells compared with LNCaP neo cells. As shown in Fig. 2C, whereas rPDGF D was processed mostly to a M, 25,000 fragment after 4 h of incubation with LNCaP-neo cells, it was more effectively processed into growth factor domains (M, 25,000, 20,000, and 15,000) in the presence of LNCaP-PDGF D cells. This suggests that proteinase expression or its activation, responsible for PDGF D processing, was up-regulated by constitutive activation of PDGF D signaling in LNCaP-PDGF D cells.

To test whether the processed forms of PDGF D are biologically active, serum-starved NIH3T3 cells were treated with CM collected from LNCaP-neo and LNCaP-PDGF D. The β-PDGFR protein was immunoprecipitated with an anti β-PDGFR Ab, and the active form was detected by immunoblot analysis using an anti-phosphotyrosine Ab. As a positive control, serum-starved NIH3T3 cells were also immunoprecipitated with an anti β-PDGFR Ab, and the active form was readily activated by PDGF BB protein treatment for 10 min, whereas no significant activation was detected with LNCaP CM. To further confirm β-PDGFR activation, we also examined activation of its downstream signaling molecule, ERKs. As shown in Fig. 3B, ERKs were phosphorylated on LNCaP-PDGF D CM treatment as effectively as PDGF BB treatment, whereas LNCaP-neo CM had little effect on ERK phosphorylation. These results demonstrate that the growth factor domain of PDGF D produced by LNCaP-PDGF D cells can activate its cognate receptor, β-PDGFR, transducing its downstream signaling pathway.

**PDGF D Enhances LNCaP Cell Proliferation.** To assess the in vitro mitogenic activity of PDGF D through an autocrine mechanism, we examined the growth rates of LNCaP-neo and LNCaP-PDGF D under normal growth conditions. During the first 24 h of culture, neither LNCaP-neo nor LNCaP-PDGF D significantly increased their cell numbers. However, drastic differences in cell numbers between LNCaP-neo and LNCaP-PDGF D were observed after the medium was conditioned by these cells for 48 h. Whereas LNCaP-neo cells barely doubled the cell number, LNCaP-PDGF D exhibited a 3–4-fold increase in cell numbers after 48 h in normal culture medium (Fig. 4), showing that PDGF D expression in prostate carcinoma cells has growth-promoting activity through an autocrine mechanism.

**PDGF D Accelerates Early Onset of Prostate Tumor Growth and Stimulates Prostate Carcinoma Cell Interactions with Stromal Cells in a Mouse Model.** The above-mentioned studies showed that tumor cell-derived PDGF D enhances tumor cell growth in an autocrine manner. These effects may be transient in vitro or may result in profound differences in vivo for tumorigenicity and/or their interactions with stromal cells that express PDGFRs. To examine the in vivo effects of PDGF D expression in human prostate carcinoma, LNCaP-neo and LNCaP-PDGF D cells were injected s.c. into the flank of male SCID mice (1 injection/animal). When the formation of tumor nodule was examined in mice sacrificed 2 weeks after injection, only one of five injections developed from LNCaP-neo cells, whereas five of five animals developed lesions from LNCaP-PDGF D cells (20% versus 100%; Fig. 5A). To further examine the onset of tumor nodules and their growth, LNCaP-neo and LNCaP-PDGF D cells were s.c. injected into both the right and left flanks (2 injections/animal) of an additional six animals, and tumor incidence and size were monitored at weekly intervals. The differences in tumor incidence between LNCaP-neo and LNCaP-PDGF D became less significant after 5 weeks (80% versus 100%; Fig. 5A), and average tumor volume was similar between these two cell lines (Fig. 5B). These results indicate that whereas the onset of tumor nodules was shortened by 10–14 days in LNCaP-PDGF D tumors compared with LNCaP-neo tumors, overall tumor incidence and tumor volume were similar between these two cell lines.

To examine the effects of tumor cell-derived PDGF D expression on host stromal cell responses in vivo, LNCaP-neo and LNCaP-PDGF D tumor tissues were harvested 5 weeks after injection, sectioned, and stained with H&E. Histological examination of LNCaP-neo tumors revealed a solid tumor mass that is well capsulated and separated from mouse stromal tissue (Fig. 6A). In contrast, LNCaP-PDGF D tumors showed a close interaction with the mouse stromal cells (Fig. 6B), suggesting a role for PDGF D in close reciprocal interplay between tumor cells and local mesenchymal cells. Among two different sections for each of eight individual LNCaP-neo and LNCaP-PDGF D

Fig. 4. Platelet-derived growth factor D accelerates cell growth in an autocrine manner. Cells (6000 cells/well) were plated in a 96-well plate, and the relative cell number of LNCaP-PDGF D (●) and LNCaP-neo (○) was determined using the 4-(3-carboxyramino)-2-(4-nitrophenyl)-2H-5-tetrazolium-1–3 benzene disulfonate (WST) assay (Roche Diagnostics GmbH, Mannheim, Germany) at the indicated time points.

Fig. 3. LNCaP-processed platelet-derived growth factor (PDGF) D is biologically active. A, serum-starved NIH3T3 cells were stimulated with 5 ml of conditioned medium (CM) from either LNCaP-PDGF D (Lanes 3 and 6) or LNCaP-neo (Lanes 4 and 7) for 10 or 60 min. Serum-starved NIH3T3 cells stimulated with 5 ml of serum-free medium supplemented with 25 ng/ml PDGF BB served as a positive control (Lane 1), and cells stimulated with serum-free medium (SF) served as a negative control (Lanes 2 and 5). β-PDGFR receptor was immunoprecipitated from 250 μg of lysate, as described in “Materials and Methods.” Resultant immunoprecipitates were resolved by reducing SDS-PAGE and analyzed by immunoblot with the indicated antibodies (Abs). The blot was immunoblotted with an Ab that recognizes both active and inactive forms of ERK1/2.

B, serum-starved NIH3T3 cells were stimulated with CM collected from LNCaP-PDGF D (Lane 3) or LNCaP-neo (Lane 4) were resolved on reducing SDS-PAGE, and the immunoblot was analyzed using an Ab specific to active extracellular signal-regulated protein kinase (ERK) 1/2 (pERK1/2). To determine basal levels of ERK1/2, the same blot was stripped and immunoblotted with an Ab that recognizes both active and inactive forms of ERK1/2.

Fig. 2. LNCaP-PDGF D cells have growth-promoting activity through an autocrine mechanism. A, to assess the potential oncogenic activity of PDGF D in prostate carcinoma, LNCaP-PDGF D and LNCaP-neo cells were observed after the medium was conditioned by these cells for 48 h. Whereas LNCaP-neo cells barely doubled the cell number, LNCaP-PDGF D exhibited a 3–4-fold increase in cell numbers after 48 h in normal culture medium (Fig. 4), showing that PDGF D expression in prostate carcinoma cells has growth-promoting activity through an autocrine mechanism.

**PDGF D Enhances LNCaP Cell Proliferation.** To assess the in vitro mitogenic activity of PDGF D through an autocrine mechanism,
xenografts examined, all of the LNCaP-PDGF D sections, but none of the LNCaP-neo sections, showed drastically increased carcinoma interactions with the stromal cells, an event known to be critical for cancer progression leading to metastasis. It should be mentioned that the average weight of xenografts formed by LNCaP-neo and LNCaP-PDGF D was comparable at the time of histological examination (1.5 versus 1.3 g, respectively), suggesting that PDGF D-mediated stromal-epithelial interactions are not directly associated with its growth-promoting activity.

We next investigated whether PDGF D stimulates prostate fibroblast cell migration in a paracrine manner and/or promotes migrative phenotype of human prostate cancer cells in an autocrine manner. As shown in Fig. 7A, cell motility of human prostate fibroblasts 1532-FTX was greatly induced in the presence of CM collected from LNCaP-PDGF D compared with treatment with CM collected from LNCaP-neo. To ensure that LNCaP-PDGF D CM-induced 1532-FTX migration does not result from increased cell proliferation rates, the wound migration assay was performed using cells pretreated with mitomycin C, a cell cycle blocker at the S phase (15). We then quantitated cell motility by a migration assay using a Transwell chamber. Whereas <2-fold induction of 1532-FTX cell migration was observed in the presence of LNCaP-neo CM compared with the serum-free medium condition, ~10-fold induction was detected in the presence of LNCaP-PDGF D CM (Fig. 7B). These results suggest that PDGF D-mediated stromal-prostate carcinoma interactions involve migration of surrounding mesenchymal cells in a paracrine manner.

When cell motility of LNCaP-neo and LNCaP-PDGF D was examined by a Transwell chamber assay (Fig. 8) as well as wound healing assay (data not shown), no significant difference was detected, suggesting that PDGF D expression has little effect on human prostate epithelial cell motility in an autocrine manner. Next, we asked whether prostate epithelial cell-produced PDGF D in the presence of prostate fibroblasts induces epithelial cell migration. To this end, cell migration of LNCaP-neo and LNCaP-PDGF D was examined by a Transwell chamber assay in the presence of prostate 1532-FTX fibroblasts on the bottom of the wells. Importantly, migration of LNCaP-PDGF D was induced significantly compared with migration of LNCaP-neo in the presence of 1532-FTX cells (Fig. 8). Taken together, these results suggest that prostate carcinoma-produced PDGF D induces PDGFR-mediated signaling transduction pathways in stromal cells, resulting in secretion of chemotactic molecule(s), which, in turn, induce prostate cell motility in a secondary paracrine manner.

**DISCUSSION**

A causative role for PDGF in tumorigenesis was suggested when v-sis (the oncogene of simian sarcoma virus) was found to be 92% homologous to PDGF-B (6, 16, 17). Consistently, studies during the past two decades have clearly indicated the significance of PDGF in human tumors including glioma (18–20), dermatofibrosarcoma (21, 22), neurofibroma (23), myelomonocytic leukemia (24), and osteo-
was restricted to the breast epithelium and tumor cells, whereas membranous PDGFR immunostaining was detected in stromal cell populations in all of the breast tissues examined (32). PDGFR staining was particularly localized in the periepithelial stroma of breast carcinomas, suggesting a paracrine stimulation of adjacent stromal tissue by breast tumor cells (32). Similar to breast cancer, paracrine mechanisms were also observed in colorectal cancer and small cell lung carcinoma (35, 36).

The present study reveals the potential oncogenic activity of PDGF D-mediated β-PDGFR in human prostate cancer in both autocrine and paracrine manners. In agreement with our hypothesis, previous studies showed that approximately 80% of prostate tumor tissues express PDGFRs at both primary and metastasized sites (7), and inhibition of PDGFR signaling using Gleevec significantly reduces prostate cancer bone metastasis in a mouse model (8). Interestingly, whereas increased expression levels of α-PDGFR and its cognate ligand, PDGF A, were detected in prostate intraepithelial neoplasia and adenocarcinoma, only β-PDGFR (and not its ligand, PDGF B) was expressed in advancing stages of prostate cancer (37, 38). These prostate cancer cells were shown to exhibit phenotypes typical to β-PDGFR activation, suggesting that an alternative ligand, besides PDGF B, may play a critical role for β-PDGFR signaling during the progression of prostate cancer. Recent discovery of PDGF D as a specific activator of β-PDGFR and our present study suggest the involvement of PDGF D in human prostate cancer development and/or progression.

Many investigators have reported a more potent transforming signal by β-PDGFR than the signal transduced by α-PDGFR (26, 39, 40). Although both PDGF AA and BB are strong mitogens, only PDGF BB can induce phenotypic transformation in murine fibroblasts. A fundamental question in cell signaling is “how do two structurally related cell surface receptor tyrosine kinases (α- and β-PDGFRs) mediate differential cellular processes often using overlapping signaling pathways?” To address this issue, we previously established NIH3T3 clones in which α-PDGFR signaling is inhibited by a dominant-negative α-PDGFR or an antisense construct of α-PDGFR (41). We showed that inhibition of α-PDGFR signaling enhanced PDGF BB-mediated phenotypic transformation, suggesting that α-PDGFR antagonizes β-PDGFR-induced transformation. Whereas both α- and β-receptors effectively activate ERKs, α-PDGFR, but not β-PDGFR, activates stress-activated protein kinase-1c-Jun NH₂-terminal kinase-1 (JNK-1). Inhibition of JNK-1 activity using a dominant-negative JNK-1 mutant markedly enhanced PDGF BB-mediated anchorage-independent cell growth, demonstrating an an-

POTENTIAL ONCOGENIC ACTIVITY OF PDGF D

In vitro, overexpression of the v-sis oncogene product (p28v-sis) or PDGF B in cells that express their receptors enhances transformation, indicating an autocrine mechanism in tumorigenesis (26, 27). In addition to the autocrine mechanism, recent studies revealed a critical role for paracrine PDGF signaling in carcinogenesis involving epithelial-stromal interactions. Using nude mice, it was demonstrated that PDGFR activation of stromal cells results in tumorigenic conversion of immortal human keratinocytes (28). Mounting evidence suggests that aberrant interaction between stroma and epithelium is critical for the neoplastic progression of breast epithelium (29, 30), and PDGF was suggested to be among the critical mediators (31–34). Expression of PDGF B-protein and mRNA was restricted to the breast epithelium and tumor cells, whereas membranous PDGFR immunostaining was detected in stromal cell populations in all of the breast tissues examined (32). PDGFR staining was particularly localized in the periepithelial stroma of breast carcinomas, suggesting a paracrine stimulation of adjacent stromal tissue by breast tumor cells (32). Similar to breast cancer, paracrine mechanisms were also observed in colorectal cancer and small cell lung carcinoma (35, 36).

The present study reveals the potential oncogenic activity of PDGF D-mediated β-PDGFR in human prostate cancer in both autocrine and paracrine manners. In agreement with our hypothesis, previous studies showed that approximately 80% of prostate tumor tissues express PDGFRs at both primary and metastasized sites (7), and inhibition of PDGFR signaling using Gleevec significantly reduces prostate cancer bone metastasis in a mouse model (8). Interestingly, whereas increased expression levels of α-PDGFR and its cognate ligand, PDGF A, were detected in prostate intraepithelial neoplasia and adenocarcinoma, only β-PDGFR (and not its ligand, PDGF B) was expressed in advancing stages of prostate cancer (37, 38). These prostate cancer cells were shown to exhibit phenotypes typical to β-PDGFR activation, suggesting that an alternative ligand, besides PDGF B, may play a critical role for β-PDGFR signaling during the progression of prostate cancer. Recent discovery of PDGF D as a specific activator of β-PDGFR and our present study suggest the involvement of PDGF D in human prostate cancer development and/or progression.

Many investigators have reported a more potent transforming signal by β-PDGFR than the signal transduced by α-PDGFR (26, 39, 40). Although both PDGF AA and BB are strong mitogens, only PDGF BB can induce phenotypic transformation in murine fibroblasts. A fundamental question in cell signaling is “how do two structurally related cell surface receptor tyrosine kinases (α- and β-PDGFRs) mediate differential cellular processes often using overlapping signaling pathways?” To address this issue, we previously established NIH3T3 clones in which α-PDGFR signaling is inhibited by a dominant-negative α-PDGFR or an antisense construct of α-PDGFR (41). We showed that inhibition of α-PDGFR signaling enhanced PDGF BB-mediated phenotypic transformation, suggesting that α-PDGFR antagonizes β-PDGFR-induced transformation. Whereas both α- and β-receptors effectively activate ERKs, α-PDGFR, but not β-PDGFR, activates stress-activated protein kinase-1c-Jun NH₂-terminal kinase-1 (JNK-1). Inhibition of JNK-1 activity using a dominant-negative JNK-1 mutant markedly enhanced PDGF BB-mediated anchorage-independent cell growth, demonstrating an an-
agonistic role for α-PDGFR-activated JNK-1 in PDGF-induced transformation. These results revealed a striking feature of PDGF signaling: the specificity and the strength of the PDGF growth signal is modulated by α-PDGFR-mediated simultaneous activation of growth-stimulatory and -inhibitory signals. With the discovery of new PDGF ligands, it has become clear that PDGFR signaling is finely growth-stimulatory and -inhibitory signals. With the discovery of new PDGF ligands, it has become clear that PDGFR signaling is finely

modulated not only by PDGF A activation of α-PDGFR and PDGF B activation of both α- and β-PDGFR, but also by PDGF C activation of α-PDGFR and PDGF D activation of β-PDGFR. Recently, it was shown that PDGF DD dimerizes αβ-PDGFR, not αα-PDGFR, but it can activate α-PDGFR through its dimerization with β-PDGFR \(\alpha\beta\)-PDGFR \((9,10)\). Thus, for better understanding of roles of PDGF signaling during human cancer progression, it would be of particular importance to unveil signal transductions pathways and oncogenic activities induced by PDGF DD in comparison with better characterized pathways induced by PDGF BB, which activates all three forms of PDGFRs (\(\alpha\alpha, \alpha\beta,\) and \(\beta\beta\)).

The unique structure of PDGF D, which requires proteolytic cleavage of the CUB domain to allow the growth factor domain to activate β-PDGFR, implements another level of regulation in PDGF signaling. The present study demonstrates that human prostate carcinoma LNCaP is capable of processing full-length PDGF D into a biologically active PDGF ligand, but that its interaction with co-receptor-like proteins may alter PDGFR signaling pathways. Considering its sequence similarities with epidermal growth factor-like protein (Uegf), and bone morphogenetic protein 1 (Bmp1; Ref. 42), the CUB domain is found in several domains of the complement subcomponents C1r/C1s, urchin epi-

localization. Conversely, the CUB domain could facilitate activation of PDGF DD by interacting with a specific protein(s) near the cell surface, localizing PDGF DD in appropriate association with the protease(s); only on this association would the protease be able to activate PDGF DD. It is also possible that the CUB domain may not serve as a mere “anchor” but that its interaction with co-receptor-like proteins may alter PDGFR signaling pathways. Considering its sequence similarities with epidermal growth factor-like protein and bone morphogenetic protein 1, it may be also worthy of investigation to determine whether the CUB domain alone after proteolytic cleavage from the full-length PDGF D has biological activity. In relation to prostate cancer, the CUB domain’s homology with bone morphogenetic protein (a protein known to play role in bone growth) may have implications in prostate cancer metastasis to the bone. This is worthy of further investigation using in vivo models.

Recent reports showed that PDGF D induces phenotypic transformation of NIH3T3 cells in vitro and also promotes tumor formation in mice (12, 46). In this study, we show that although PDGF D promotes human prostate carcinoma cell growth in vitro, it accelerates tumor formation only at the early stages, with little effect on the overall tumor incidence or the size of the tumors in vivo. However, PDGF D drastically increased the ability of human prostate carcinoma cells to interact with host stromal components in mice, an event known to be critical for prostate cancer progression. Our in vitro studies suggest that PDGF D is sufficient to induce migration of fibroblasts but that PDGF D alone is not sufficient to induce epithelial migration. Rather, PDGF D induces an as yet unidentified signal from the fibroblasts, which then induces the epithelial cell migration in a secondary paracrine manner. Taking our results together with those of recent studies reporting the significance of PDGF signaling in the progression of prostate cancer as well as β-PDGFR signaling in metastasis, we propose that prostate carcinoma cells auto-activate PDGF D and use its sig-

netic protein (a protein known to play role in bone growth) may have unique regulatory functions toward PDGF D activity in vivo. In vitro, it accelerates tumor formation in a mouse model of experimental prostate cancer – PDGF signaling in metastasis, we propose that prostate carcinoma cells auto-activate PDGF D and use its sig-

ACKNOWLEDGMENTS

We thank Dr. R. Fridman and Pam Osenkowski for providing technical assistance with the vaccinia virus system.

REFERENCES


A Potential Oncogenic Activity of Platelet-Derived Growth Factor D in Prostate Cancer Progression

Carolyn V. Ustach, Marcus E. Taube, Newton J. Hurst, Jr., et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/5/1722

Cited articles
This article cites 46 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/5/1722.full.html#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
/content/64/5/1722.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.