PC Cell-Derived Growth Factor Expression in Prostatic Intraepithelial Neoplasia and Prostatic Adenocarcinoma

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ABSTRACT

Purpose: PCDGF (PC cell-derived growth factor), also called progranulin, is a M_r 88,000 glycoprotein precursor of granulin. It is a novel growth factor that stimulates cell proliferation, confers epithelial tumorigenesis, and promotes tumor invasion. Here we investigate the potential of PCDGF as a therapeutic target for prostate cancer.

Experimental Design: We studied the expression of PCDGF in invasive prostate cancer, adjacent high-grade prostatic intraepithelial neoplasia (PIN), and benign prostate tissue from 99 human prostate specimens. The level of PCDGF expression was correlated with various clinicopathological characteristics.

Results: Normal prostate tissue did not express (53/99), or expressed low levels (46/99) of PCDGF. In the 46 normal prostate specimens that expressed PCDGF, most of them had less than 10% of cells expressing PCDGF. PCDGF expression could be detected in more than 50% of cells in all specimens of PIN and invasive prostate cancer. The expression of PCDGF in normal prostate tissue was much less intense and in a smaller fraction of cells than in PIN and invasive adenocarcinoma ($P < 0.0001$). There was no correlation of PCDGF expression with age, Gleason score, pathological stage, status of lymph node metastasis, extraprostatic extension, perineural invasion, surgical margins, and vascular invasion.

Conclusions: Our data suggest that the induction of PCDGF expression occurs during the development of PIN. PCDGF may be a new molecular target for the treatment and prevention of prostate cancer.

INTRODUCTION

Progranulin, also known as PC cell-derived growth factor (PCDGF) and its proteolytic product granulin, also called epithelin, belong to a novel class of growth factors (1–3). These growth factors stimulate cell proliferation and promote anchorage-independent growth (4). PCDGF is expressed at high levels in the rapidly proliferating cells, such as skin cells, deep crypts of gastrointestinal tract, and immune cells. However, it is expressed at low levels in the cells that are not mitotically active, such as muscular and liver cells (5). In cells deficient of insulin-like growth factor I receptor, PCDGF potently stimulates cell proliferation and is the only known growth factor that circumvents the blockade of the cell cycle imposed by this deficiency (6). PCDGF is also involved in the normal development of early embryonic epithelia (7).

At the molecular level, PCDGF directly activates mitogen-activated protein kinase, phosphatidylinositol 3′-kinase, and focal adhesion kinase signaling pathways (4). In 3T3 mouse embryo fibroblasts deficient in insulin-like growth factor I receptor, PCDGF overcomes this deficiency and stimulates cell proliferation via the activation of the mitogen-activated protein and phosphatidylinositol 3′-kinase pathways (8). It also increases cyclin D1 expression accompanied by increased pRB phosphorylation in breast cancer cells (9). Granulin interacts with HIV Tat and cyclin T1 to regulate gene transcription (10).

PCDGF is important in tumorigenesis (4). It has also been found to be expressed at a higher level in highly aggressive PC teratoma cells when compared with matched controls (3). Moreover, its expression is very low in nontransformed MCF-10A cells, a human mammary epithelial cell line, but higher in tumor-derived cells, and highest in estrogen receptor-negative cancer cells (11). PCDGF mediates estrogen-mediated mitogenic activity of breast cancer cells. Its overexpression results in cell proliferation in the absence of estrogen and makes cells resistant to tamoxifen. Cells transfected with antisense PCDGF expression vector have a 74% decrease in cell proliferation when compared with cells transfected with vector alone (9). The expression of PCDGF is also increased in invasive ovarian carcinoma and multiple myeloma cells (12, 13).

In this study, we evaluated PCDGF expression in invasive prostate cancer, its adjacent high-grade PIN and normal prostate tissue, and correlated its expression with other clinicopathological characteristics.

MATERIALS AND METHODS

Patients. Invasive prostate cancer specimens ($n = 99$) including adjacent PIN and normal prostate tissues from 1990

Table 1 Patient characteristics and prostate cancer cell-derived growth factor (PCDGF) expression in prostate cancer

Patient characteristic	% of total patients ($n = 99$)	Mean % of cells staining with PCDGF antibody ($\pm$SD)	Mean PCDGF [a] antibody staining intensity ($\pm$SD)
Primary Gleason grade			
2	13	91 $\pm$ 7	2.5 $\pm$ 0.5
3	46	90 $\pm$ 10	2.6 $\pm$ 0.5
4	27	90 $\pm$ 8	2.4 $\pm$ 0.5
5	13	91 $\pm$ 8	2.6 $\pm$ 0.5
Secondary Gleason grade			
2	15	89 $\pm$ 8	2.4 $\pm$ 0.5
3	35	91 $\pm$ 7	2.5 $\pm$ 0.5
4	35	88 $\pm$ 11	2.6 $\pm$ 0.5
5	14	93 $\pm$ 5	2.6 $\pm$ 0.5
Gleason sum			
<7	30	91 $\pm$ 7	2.5 $\pm$ 0.5
7	41	88 $\pm$ 11	2.6 $\pm$ 0.5
>7	28	91 $\pm$ 6	2.6 $\pm$ 0.5
Tumor classification			
T_{2a}	11	91 $\pm$ 7	2.5 $\pm$ 0.5
T_{2b}	46	90 $\pm$ 8	2.6 $\pm$ 0.5
T_{3a}	26	90 $\pm$ 10	2.4 $\pm$ 0.5
T_{3b}	16	90 $\pm$ 7	2.5 $\pm$ 0.5
Lymph node metastasis			
Positive	14	88 $\pm$ 13	2.5 $\pm$ 0.5
Negative	85	90 $\pm$ 8	2.3 $\pm$ 0.5
Extraprostatic extension			
Positive	59	90 $\pm$ 8	2.6 $\pm$ 0.5
Negative	40	90 $\pm$ 9	2.5 $\pm$ 0.5
Surgical margin			
Positive	58	90 $\pm$ 7	2.5 $\pm$ 0.5
Negative	41	90 $\pm$ 10	2.6 $\pm$ 0.5
Vascular invasion			
Positive	29	90 $\pm$ 9	2.4 $\pm$ 0.5
Negative	70	90 $\pm$ 8	2.6 $\pm$ 0.5
Perineural invasion			
Positive	86	90 $\pm$ 9	2.5 $\pm$ 0.5
Negative	13	90 $\pm$ 7	2.5 $\pm$ 0.5
High-grade PIN			
Positive	95	81 $\pm$ 12	2.3 $\pm$ 0.6
Negative	4	90 $\pm$ 8	2.5 $\pm$ 0.5

[a] PCDGF, PC cell-derived growth factor; PIN, prostatic intraepithelial neoplasia.

through 1995 were obtained from the surgical pathology files of Indiana University Medical Center. Standard sections from radical prostatectomy specimens were prepared for histological examination. The patients ranged in age from 44 to 77 years (mean, 63 years). Grading of the primary tumor from radical prostatectomy specimens was performed according to the Gleason system (14). The Gleason score ranged from 4 to 10 (Table 1). Almost one-third (30/99) had Gleason scores less than 7. A similar number (28/99) had Gleason scores greater than 7. Pathological staging was performed according to the 1997 TNM classification (tumor-lymph node-metastasis; Ref. 15). Less than 15% (14/99) had lymph node metastases, and nearly one-third (29/99) had vascular invasion. This research was approved by the Indiana University Institutional Review Board.

Immunohistochemistry. Serial 5-µm-thick sections of formalin-fixed paraffin-embedded slices of prostate cancer specimens were used for the study. Tissue blocks that contained the maximum amount of tumor and highest Gleason score were selected. One representative slide from each case was analyzed. We recognized the limitation of sample variation. Slides were deparaffinized in xylene twice for 5 min and rehydrated through graded ethanol solution to distilled water. Antigen retrieval was carried out by heating sections in a citrate-buffer (pH 6.0; DAKO Corporation, Carpintera, CA) for 20 min. Endogenous peroxidase activity was inactivated by incubation in 3% H_2O_2 for 15 min. Nonspecific binding sites were blocked using Protein Block (DAKO Corp) for 20 min. Tissue sections were then incubated with the 6B2 murine monoclonal antibody against human PCDGF (1:200 dilution; A&G Pharmaceuticals Inc., Columbia, MD) for 1 h at room temperature, followed by biotinylated secondary antibody (DAKO Corp.), and peroxidase-labeled streptavidin. 3,3-diaminobenzidine was used as the chromogen in the presence of hydrogen peroxide. Positive and negative controls were run in parallel with each batch, and appropriate results were obtained.

Evaluation of PCDGF Expression. The extent and intensity of PCDGF staining were evaluated in benign epithelium, PIN, and adenocarcinoma from the same slide for each case. Microscopic fields with the highest degree of immunoreactivity were chosen for analysis. At least 1000 cells were analyzed in
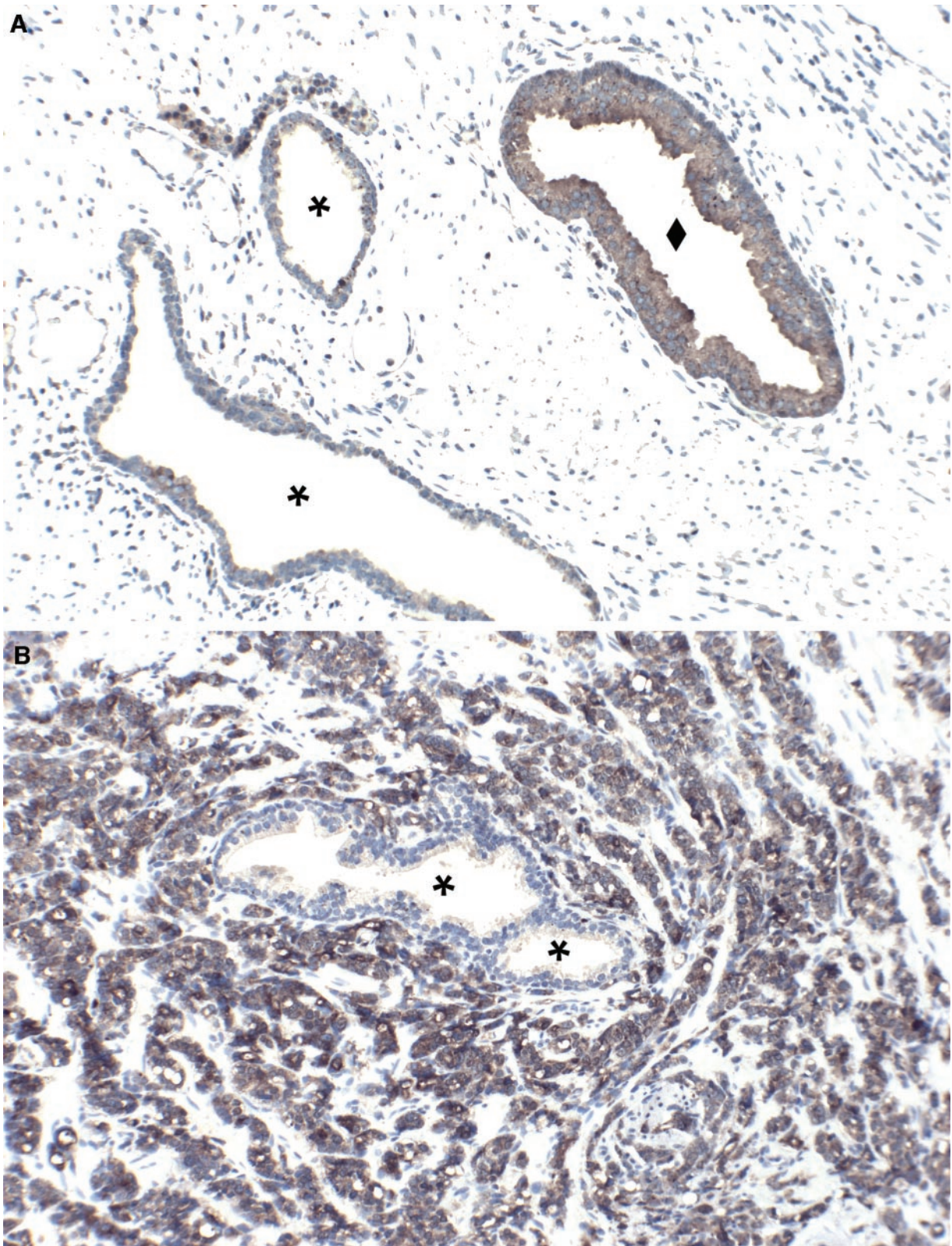
Fig. 1 Prostate cancer cell-derived growth factor (epithelin/granulin precursor) expression was elevated in high-grade prostatic intraepithelial neoplasia (*A*) and prostatic adenocarcinoma (*B*). ◆, prostatic intraepithelial neoplasia; ✶, adjacent normal glands.

Table 2 The intensity of prostate cancer cell-derived growth factor (PCDGF) expression in normal tissue, high-grade prostatic intraepithelial neoplasia (PIN), and prostatic adenocarcinoma

	Intensity of PCDGF expression			
Tissue	0 (%)	1 (%)	2 (%)	3 (%)
Normal	53 (54)	46 (46)	0	0
High-grade PIN	0	1 (1)	51 (52)	47 (48)
Invasive cancer	0	0	45 (46)	54 (54)

each case. The percentage of cells exhibiting staining in each case was evaluated semiquantitatively on a 5% incremental scale ranging from 0 to 95%. A numeric intensity score was set from 0 to 3 (0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining). Adjacent inflammation and atrophy did not appear to interfere with PCDGF staining. These methods follow the methods of our previous work (16–19).

Statistical Analysis. The mean percentage of immunoreactive cells in benign epithelium, high-grade PIN, and adenocarcinoma were compared using the Wilcoxon paired signed-rank test. The intensity of staining for PCDGF in benign epithelium, high-grade PIN, and adenocarcinoma were compared using Cochran-Mantel-Haenszel tests for correlated ordered categorical data. A $P < 0.05$ was considered significant, and all Ps were two-sided.

RESULTS

Significant difference in PCDGF expression was observed between normal prostate tissue, high-grade PIN, and invasive cancer (Fig. 1; Tables 2 and 3). PCDGF was not expressed in 54% (53/99) of the normal prostate tissue. In the remaining 46 specimens, it was expressed at low levels in normal tissue, with a staining intensity of 1+. In 78% (36/46) of these 46 specimens, no more than 10% of cells expressed PCDGF. The mean percentage cells expressing PCDGF was 5%.

PCDGF was expressed at high levels in PIN and invasive prostate cancer. PCDGF could be detected in all of the PIN specimens and invasive cancer specimens. Ninety-eight of 99 PINs and all of the invasive cancer specimens had intermediate or high levels of PCDGF (staining intensity of 2+ or 3+). Fifty-one of 99 PINs *versus* 45 of 99 invasive cancers had intensity scores of 2+. Forty-seven of 99 PINs *versus* 54 of 99 carcinomas had staining intensities of 3. In 57% (56 of 99) of PINs and 78% (77 of 99) of invasive prostate cancer specimens, 90% or more of the cells had PCDGF immunoreactivity. All 99 specimens of PIN and invasive prostate cancer had 50% or more cells expressing PCDGF. The mean percentage of PCDGF staining was 84% for PIN and 90% for invasive cancer. The difference of expression between PIN and invasive cancer was not statistically significant ($P = 0.10$). When compared with normal prostate tissue, both the intensity and the fraction of cells expressing PCDGF were significantly greater in PIN and invasive cancer ($P < 0.0001$).

After finding that PCDGF is expressed at high levels in PIN and invasive cancer, we tested whether the level of PCDGF expression is related to other clinicopathological parameters. There was significant concordance of PCDGF expression between PIN and invasive cancer. Forty-two of the 50 PIN that had high levels of PCDGF expression also had high levels of PCDGF in the invasive cancer cells in the same specimens. Of the 47 invasive cancer specimens that expressed intermediate levels of PCDGF (staining intensity of 2+), 38 also had lower expression in the adjacent PINs. There was no correlation of PCDGF expression with other clinicopathological characteristics: patient's age, Gleason score, pathological stage, status of lymph node metastasis, extraprostatic extension, perineural invasion, surgical margins, and vascular invasion.

DISCUSSION

PCDGF stimulates cell proliferation and promotes tumor formation *in vitro* and *in vivo*. Here our data suggest that PCDGF may also be important in the progression of prostate cancer. The induction of PCDGF expression seems to be an early event in the evolution of prostate cancer.

PCDGF overexpression may confer a proliferation advantage in the cells of PIN and invasive prostatic adenocarcinoma. Our study showed that PCDGF was not expressed, or was expressed at low levels, in normal prostate tissue, but was expressed at high levels in almost all PIN and invasive prostate cancer. As shown in previous studies, PCDGF overcomes the mitotic block in insulin-like growth factor I receptor-deficient cells (6). Overexpression of PCDGF makes breast cancer cells become hormone independent and tamoxifen resistant, and less growth restricted by the surrounding matrix (9). Inhibition of PCDGF significantly decreases cell proliferation (11). Therefore, it is possible that PCDGF overexpression may provide a proliferation advantage for PIN and invasive cancer cells over the adjacent normal prostate tissue and may contribute to tumorigenesis of prostate cancer. Further investigation will be needed to determine whether PCDGF expression is involved in the development of androgen independence of the prostate cancer.

Evidence support the contention that PCDGF overexpression may also promote cancer invasion and metastasis. First, malignant cells must be able to escape the restriction of the extracellular matrix. Second, the escaped cells must be able to survive without attachment to their normal substrate and to proliferate in this environment. Overexpression of PCDGF overcomes the inhibition of cell growth imposed by the surrounding interstitial type I collagen. It increases the production of several matrix-degrading enzymes, such as matrix metalloproteinase 13 and 17 in SW-3 adrenal carcinoma cells (20). These matrix-degrading enzymes, especially matrix metalloproteinase-13, are strongly associated with several invasive cancers (21). These cells also become independent of exogenous hormone stimulation. PCDGF-expressing cells can escape anoikis, a form of cell

Table 3 The fraction of cells stained positive for prostate cancer cell-derived growth factor expression

Tissue	Staining (%)	Mean staining (SD)	Range
Normal	46	5.3 (7.5)	0–40
High-grade PIN[a]	99	84.2 (12.6)	50–95
Invasive cancer	99	90.3 (8.3)	50–95

[a] PIN, prostatic intraepithelial neoplasia.

death of adherent cells after detachment from the normal substrate (4). All of these features constitute the property of PCDGF to be an important factor for tumor progression and metastasis. If PCDGF exhibits similar functions in prostate cancer, it is conceivable that cells with high PCDGF would be highly malignant and metastatic.

Our findings that PIN and invasive adenocarcinoma similarly overexpress PCDGF suggest that the induction of PCDGF is an early event in prostate tumorigenesis. In most cases, it appears that the induction of PCDGF expression occurs during the development of PIN. In 38 of 47 invasive cancer specimens that had intermediate levels of PCDGF, the adjacent PIN also had intermediate levels of PCDGF. This suggests that in some cells, high levels of PCDGF may not be necessary for invasion. We recognize that PCDGF is not the only growth factor at play in the transition from normal cells to neoplastic cells. Suppression of PCDGF partially inhibits, but does not completely block, cell proliferation (11). This suggests that, besides PCDGF, other signal transduction pathways exist that stimulate cell growth. This is supported by the experiments that PCDGF activates the extracellular signal-regulated kinase, phosphatidylinositol 3'-kinase and focal adhesion kinase cascades, but does not stimulate c-myc expression (4, 9). It is possible that in PIN and adenocarcinoma with intermediate levels, either other redundant pathways exist or these intermediate levels are sufficient to stimulate tumorigenesis in their corresponding cellular background.

In conclusion, we found that PCDGF is expressed at low levels or not at all in normal prostate tissue, but it is present in PIN and cancer cells. There is significant concordance on PCDGF expression between PIN and invasive cancer, suggesting that the induction of PCDGF expression is an early event of prostate carcinogenesis. PCDGF may be a novel molecular target for the treatment and prevention of prostate cancer.

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