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Coexpression of Keratinocyte Growth Factor and Its Receptor in Normal and Prostate Cancer Tissues: Possible Formation of Autonomous Andromedin Loop

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Keratinocyte growth factor (KGF), an androgen-dependent epithelial mitogen, and its receptor (KGFR) have been implicated in the regulation of cell growth and differentiation in prostate tissue. This study was designed to determine the expression and role of KGF and KGFR in normal and prostate cancer tissues, especially in relation to cell kinetics. In 41 cases of prostate cancer in paraffin-embedded specimens, the expression of KGF and KGFR at the levels of protein and mRNA was analyzed by immunohistochemistry using newly raised antibodies and *in situ* hybridization, respectively. We also examined expression of androgen receptor (AR) and Ki-67 labeling index (LI). In normal and hyperplastic prostate tissues, both KGF mRNA and protein were localized in AR positive stromal cells, while those of KGFR were localized in glandular epithelial cells. In prostate cancer, however, coexpression of KGF and KGFR was observed in 14/41 cases, and significantly correlated with high Gleason scores, bone metastasis and high Ki-67 LI. The relapse-free survival of patients suffering from prostate cancers coexpressing KGF and KGFR was significantly shorter than that of patients from the other ones. Therefore, our results indicate that coexpression of KGF and KGFR in prostate cancer may predict metastatic and proliferative activities, possibly due to the formation of an autonomous andromedin loop.

Key words: KGF, KGFR, benign prostatic hyperplasia, prostate cancer

I. Introduction

Prostate cancer is the most common male malignancy and the second leading cause of cancer-related death among men in Western countries [14]. Clinically, the involvement of sex steroids, especially androgen, in the pathogenesis and progression of prostate cancer has been well established [10]. However, the molecular mechanisms underlying the abnormal growth of prostate cancer are still controversial despite the extensive research in this area.

During the past decade, it has become apparent that the action of sex steroids in their target tissues is mediated through locally produced growth factors in an autocrine and/or paracrine fashion [11, 19, 40]. In the case of androgen in prostatic tissues, epidermal growth factor (EGF) [8], vascular endothelial growth factor (VEGF) [17, 22], fibroblast growth factor-1 (FGF-1) [27], keratinocyte growth factor (KGF) [28] and FGF-8 [18] have been considered as possible mediators. Among these growth factors, KGF seems to be the most intriguing candidate to regulate normal prostatic growth and development, because it directly stimulates proliferation of prostatic epithelial cells [40] and induces ductal branching morphogenesis [34].

KGF is a unique member of the FGF gene family, which is usually secreted from stromal cells and acts as a specific epithelial mitogen in a unidirectional paracrine fashion [5, 32]. The biologic effects of KGF are mediated via KGF receptor (KGFR or bek IIIb product), which is generated as an alternative splicing variant of FGF receptor 2 (FGFR2 or bek IIIa product) [16, 24]. Recently, it has been

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reported that the promoter region of KGF gene contains one or more androgen responsive elements [4], indicating that the transcriptional activity of KGF gene may depend upon androgen. These findings support the notion that KGF is an androgen-dependent epithelial mitogen, i.e., an andromedin [29, 34, 40].

Using RT-PCR, Leung *et al.* [21] recently reported that the expression of KGF mRNA was significantly upregulated in prostate cancer. They also showed that persistent expression of KGFR mRNA was associated with the androgen-insensitivity of prostate cancer growth. These data suggest that abnormalities in the paracrine loop consisting of androgen-dependent expression of KGF in stromal cells and KGFR expression in glandular epithelial cells might be involved in the development and progression of prostate cancer. However, for a better understanding of the KGF-KGFR loop in prostate cancer tissues, histochemical analysis of those expressions at the level of individual cells would be required because of the complicated nature of histological grading in prostate cancer, where cancer foci with different Gleason grades co-exist.

To date, most of the studies on the expression of KGF and KGFR in prostate tissues [1, 23] as well as other organs [13, 19] have been performed at the transcript level. Unfortunately, however, elevated levels of certain mRNAs do not always correlate with the production of the protein, because of the presence of translational and/or post-translational regulation [37]. Therefore, there is a substantial necessity to analyze the expression of KGF and KGFR at the protein level. To detect KGF and KGFR in the sections of prostate cancer tissues, immunohistochemistry seems to be most appropriate. However, to our knowledge, there are only a few appropriate antibodies for KGF and KGFR to allow localization of these molecules in histopathological paraffin sections. Fortunately, we have recently succeeded in raising new specific antibodies for them, and have confirmed that they work very well in paraffin-embedded sections [35, 38, 39].

In the present study, we examined the expression of KGF and KGFR proteins and mRNAs in paraffin-embedded sections of normal, hyperplastic and cancerous prostate tissues, by immunohistochemistry using our newly prepared antibodies and nonradioactive *in situ* hybridization with a high resolution using oligodeoxynucleotide (oligo-DNA) probes. We also simultaneously examined androgen receptor (AR) expression and proliferative activity in these tissues. The results indicated that KGF and KGFR co-expression correlated signigicantly with prostate cancers of high Gleason scores, indicating that such coexpression may form autonomous autocrine and/or paracrine loop in prostate cancer cells.

II. Materials and Methods

Biochemicals and chemicals

Recombinant human KGF (rh KGF) was purchased from PeproTech EC (London, UK). Recombinant human KGFR (rh KGFR) was purchased from R&D Systems Inc. (Minneapolis, MN), which was human FGFRα2 (IIIb)/Fc Chimera protein. Bovine serum albumin (BSA, minimum 99%, essentially free from fatty acids and globulins), proteinase K, yeast transfer RNA (RNA transfer, type X from baker's yeast), salmon testis DNA, dextran sulfate (MW=500,000), polyvinylpyrolidone (MW=360,000), Triton X-100, Brij 35 and 3-aminopropyltriethoxysilane were purchased from Sigma Chemical Co. (St. Louis, MO). 3,3'-diaminobenzidine/4HCl (DAB) was purchased from Dojin Chemical Co. (Kumamoto, Japan). Formamide (deionized, 99% pure, essentially free from nuclease and protease) was purchased from Nacalai Tesque (Kyoto, Japan). Ficoll 400 (MW=400,000) was purchased from Pharmacia (Peapack, NJ). All other reagents used in the present study were from Wako Pure Chemicals (Osaka, Japan) and were of analytical grade.

Prostate tissues

Cancerous prostate tissues were obtained from 41 patients by prostate needle biopsy at the Department of Urology, Nagasaki University Hospital, between 1996–2000. Sections from all specimens were stained with hematoxylin and eosin (H & E) for histopathological examination. The grade of malignancy was determined according to the Gleason grading system [3] and clinical stage was evaluated according to Whitmore-Jewett staging system [15]. Gleason scoring was evaluated by two different observers without any knowledge or the patients' clinical background. The clinicopathological data of prostate cancer patients are summarized in Table 1.

All 41 patients who received prostate needle biopsy were later treated with endocrine therapy including luteinizing hormone-releasing hormone (LH-RH) analogue with or without non-steroidal anti-androgenic agents. The initial endocrine treatment was continued until the evidence of hormone-refractory condition. Patients with hormone-refractory prostate cancer were defined as described previously [25, 33]. In addition, control prostate needle biopsy specimens were obtained from 5 patients who were later confirmed histopathologically to be negative for malignancy or prostatic intraepithelial neoplasia. Hyperplastic prostate tissues were obtained from 4 patients with benign prostatic

Table 1. *Patient characteristics at diagnosis*

Age (years)	70 (51–86)[a]
PSA (ng/ml)	82.9 (3.1–1600)
Gleason score	
5–6	8 (20)[b]
7–8	26 (63)
9–10	7 (17)
Clinical stage	
B	7 (17)
C	12 (29)
D	22 (54)

[a] Median (range), [b] Number of patients (%).

hyperplasia by suprapubic open prostatectomy. None of the patients received any treatment prior to tissue sampling. All patients gave their informed consent to the research protocol, which was approved by the local ethics committee.

For immunohistochemistry and *in situ* hybridization, all specimens were fixed with 10% neutral buffered formalin and embedded in paraffin using a standard procedure. The specimens were cut serially into 5 μm-thick sections and mounted on 3-aminopropyltriethoxysilane-coated glass slides.

Antibodies

Primary antibodies used in the present immunohistochemistry were listed in Table 2. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG F(ab)'$_2$ was purchased from Medical & Biological Laboratories (Nagoya, Japan). HRP-conjugated goat anti-mouse IgG F(ab)'$_2$ was purchased from Chemicon International (Temecula, CA). Normal goat IgG, normal rabbit IgG and normal mouse IgG were purchased from Sigma.

Western blot analysis of KGF and KGFR

Western blotting procedure was essentially the same as described by Yamayoshi *et al.* [39]. Briefly, lyophilized rh KGF and rh KGFR were reconstituted by 1% BSA in PBS to a concentration of 1 mg/ml using the protocol provided by the manufacturer. Human prostate cancer specimens were homogenized in lysis buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 0.5% Triton X-100, 10% glycerol supplemented with protease inhibitors [10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonylfluoride (PMSF)] on ice. The samples were mixed with a loading buffer (250 mM Tris-HCl [pH 6.8], 30% glycerol, 0.01% bromophenol blue, 2% SDS and 10% 2-mercaptoethanol) and boiled for 5 min. In the next step, 100 ng of both recombinant proteins and 20 μg of the sample lysates were separated by SDS-PAGE with gradient gel (Daiichi Pure Chemicals, Tokyo, Japan) of 15–25% for detection of KGF and 4–20% for detection of KGFR, and then electrophoretically transferred onto polyvinylidene difluoride membranes (PVDF) (Immobilon, Millipore Corp., Bedford, MA). The membranes were blocked in PBS with 5% nonfat dry milk and 5% BSA to reduce non-specific binding of antibodies for 1 hr. Then the membranes were incubated with 5 μg/ml anti-KGF IgG or 0.5 μg/ml anti-KGFR IgG for overnight, respectively. After washing with 0.2% Tween 20 in PBS, the membranes were reacted with HRP-conjugated goat anti-rabbit IgG, F(ab)'$_2$ at a dilution 1:2,500 for 1 hr. Finally, after washing with 0.2% Tween 20 in PBS, the bands were visualized by enhanced chemiluminescence system (Amersham, Buckinghamshire, UK).

Table 2. *List of primary antibodies for immunohistochemistry*

Antigen	Antibody	Working dilution
KGF	Polyclonal; Human KGF	20 μg/ml
KGFR	Polyclonal; Human KGFR	5 μg/ml
Ki-67	MIB-1	1:100
AR	NCL-AR-318	1:50

Polyclonal antibodies against human KGF and KGFR were raised in our laboratories in cooperation with Nichirei Co. (Tokyo, Japan). MIB-1 was purchased from Immunotech (Marseille, France). NCL-AR-318 was purchased from Novocastra Laboratories (Newcastle, UK).

Immunohistochemistry

We performed indirect enzyme-immunohistochemistry. Immunohistochemical procedure of KGF, KGFR and Ki-67 was described previously [35, 38, 39]. AR immunostaining were performed according to Ehara *et al.* [2]. Briefly, the paraffin sections were dewaxed with toluene and rehydrated by serial graded ethanol solutions. Next, in the case of KGFR, the slides were pretreated with 0.2% Triton X-100 in PBS for 15 min. In the cases of AR and Ki-67, paraffin sections were autoclaved at 120°C for 10 min in 10 mM citrate buffer (pH 6.0). After washing with PBS, endogenous peroxidase activity was inhibited by immersing the slides in 0.3% H_2O_2 in methanol for 30 min. After washing with PBS again, the slides were preincubated with 500 μg/ml normal goat IgG and 1% BSA in PBS for 1 hr to reduce non-specific binding of antibodies. Then, the sections were reacted with primary antibody listed in Table 2. After washing with 0.075% Brij 35 in PBS, the sections were incubated with HRP-labeled goat anti-rabbit IgG F(ab)'$_2$ or HRP-labeled goat anti-mouse IgG F(ab)'$_2$ at 1:100 dilution for 1 hr. After washing with 0.075% Brij 35 in PBS, the sites of HRP were visualized with DAB and H_2O_2 with or without

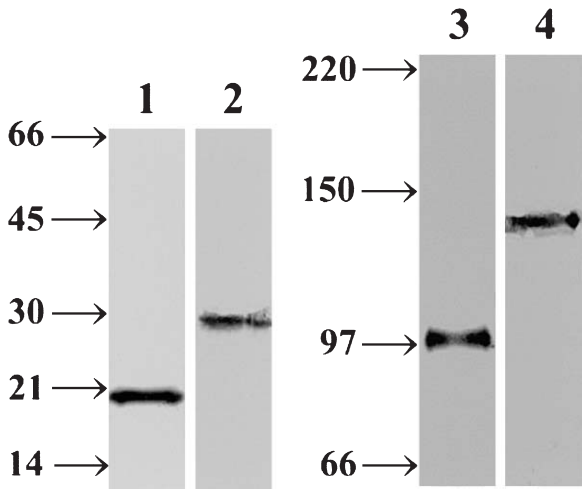

Fig. 1. Western blot analysis of KGF and KGFR. Lane 1 and 2 were analyzed by immunoblotting using anti-KGF IgG. Lane 3 and 4 were assessed by immunoblotting with anti-KGFR IgG. Lane 1, rh KGF; lane 2 and 4, extracts of Gleason grade 5 prostate cancer specimens which was positive to both KGF and KGFR by immunohistochemistry; lane 3, rh KGFR. Numbers on the left side indicate the position of molecular size markers.

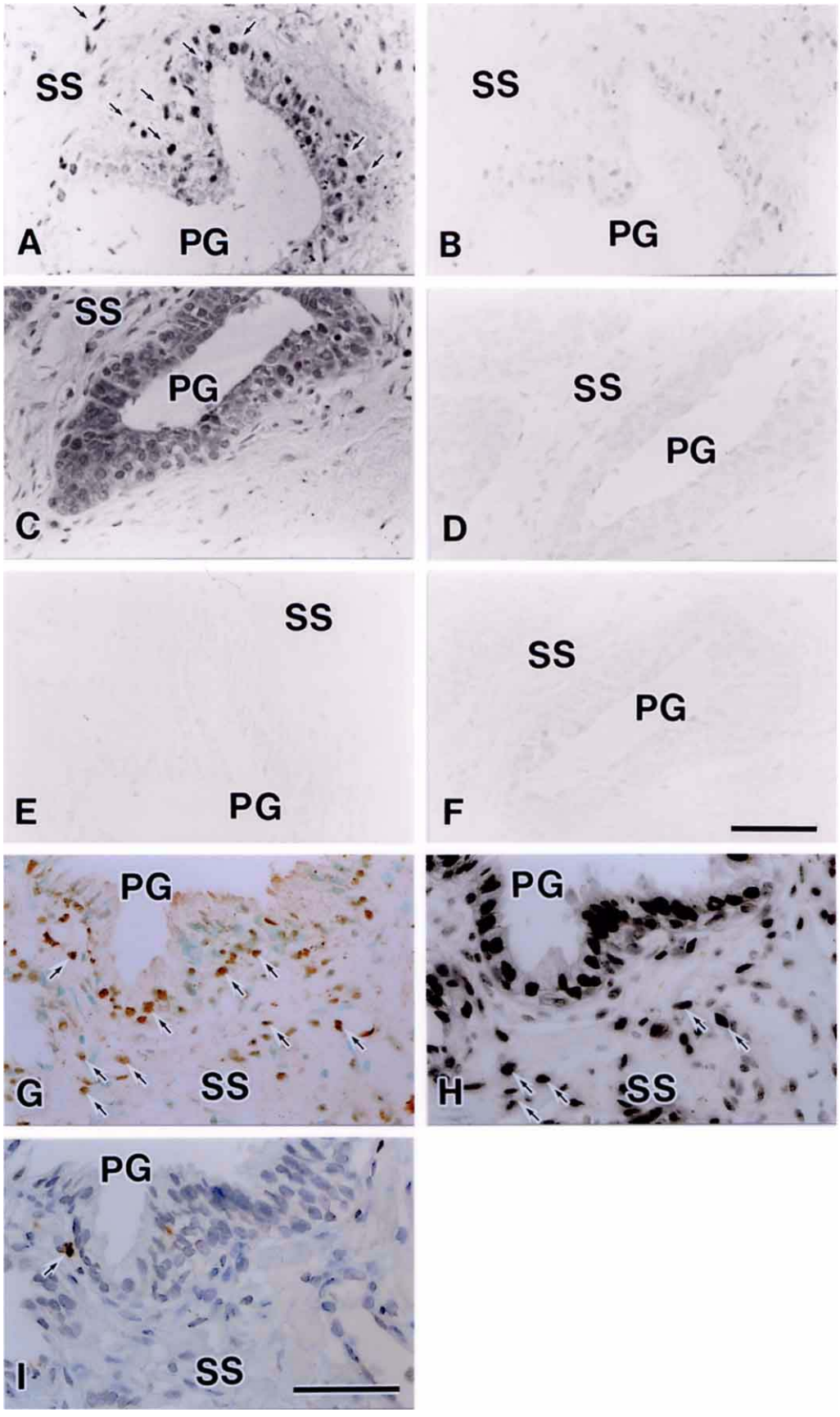

Fig. 2

Table 3. *Correlation of KGF and KGFR expression to the various prognostic parameters*

	Coexpression of KGF and KGFR (n=14)	Expression of KGF or KGFR alone (n=27)	p value
Age (years)	66.5 (51–79)[a]	72 (63–89)	NS
PSA (ng/ml)	101 (3.1–1600)	69.5 (5.2–1600)	NS
Gleason score			
5–8	5 (12)[b]	25 (60)	0.035
9–10	9 (22)	2 (6)	
Bone metastasis			
Positive	10 (24)	9 (22)	0.026
Negative	4 (11)	18 (43)	
Ki-67 LI	14.3±4.1[c]	5.7±2.6	<0.001
AR stroma			
Positive	6 (15)	11 (27)	NS
Negative	8 (19)	16 (39)	
KGF stroma			
Positive	4 (11)	8 (19)	NS
Negative	10 (24)	19 (46)	

[a] Median (range), [b] Number of patients (%), [c] Mean±SD, NS: not significant.

nickel and cobalt ions. Finally, the sections were counterstained with methyl green or Mayer's hematoxylin. As a control, some sections were reacted with normal rabbit IgG instead of the specific antibody at the same concentration. Furthermore, other sections were incubated with the primary antibody in the presence of an excess amount of synthetic oligopeptide of KGF (8-1) and KGFR (10-1) for 500-fold at a molar ratio, respectively. The staining intensities for KGF, KGFR, AR and Ki-67 were determined as positive or negative, compared to that of the background staining with normal rabbit IgG or normal mouse IgG, respectively.

In situ *hybridization*

The preparation of oligo-DNA probes for KGF and KGFR mRNAs was described previously [35, 38]. Non-radioactive *in situ* hybridization for KGF and KGFR mRNAs was performed according to the protocol reported previously with a slight modification [20, 35, 36, 38, 42]. Briefly, sections were dewaxed and rehydrated using a standard procedure. This was followed by the successive treatments with 0.2 N HCl for 20 min and 20 μg/ml proteinase K at 37°C for 15 min. After post-fixation with 4% paraformaldehyde in PBS for 5 min, the sections were immersed in 2 mg/ml glycine in PBS for 30 min and kept in 40% deionized formamide in 4×SSC until used for hybridization. Hybridization was carried out at 37°C for KGF and 42°C for KGFR with T-T dimerized antisense KGF oligo-DNA (2 μg/ml) and KGFR oligo-DNA (1 μg/ml) dissolved in the hybridization medium for 17 hr. Then, the slides were washed 3 times with 50% formamide in 2×SSC, twice with 50% formamide in 0.5×SSC at 37°C, followed by 2×SSC. After a successive treatment with ethanol and acetone to delipidate the sections, the slides were processed immunohistochemically, and the sites of peroxidase were visualized with DAB and H_2O_2 in the presence of nickel and cobalt ions. In every run, a sense probe was used as a negative control. Furthermore, to evaluate the preservation of hybridizable RNA in tissue sections, a serial section was used for 28S rRNA staining [42]. The proportion of cells positive for KGF and KGFR mRNAs was determined by an image analyzer (DAB system; Carl Zeiss, Thornwood, NY). Positive cells were determined based on the staining density over that with the sense probe.

Quantitative analysis

Quantitative analysis for AR expression in the stromal cells surrounding the cancerous regions was carried out according to Henshall *et al.* [7]. The region containing above 30% AR expressing-cells was defined as positive expression because loss of AR expression in the stromal cells surrounding the cancerous regions (≤30%) is reported to correlate with poor clinical outcome in prostate cancer. To calculate the labeling index for Ki-67, more than 1,000 cells were counted in each cancer specimen, and the number of Ki-67-

Fig. 2. Immunohistochemistry of KGF and KGFR in normal (**A–F**) and hyperplastic prostate tissue (**G–I**). (**A**) Immunohistochemical detection of KGF. (**B**) Competition test on the serial section by adding an excess amount of 8-1 synthetic peptides to anti-KGF IgG (500-fold at a molar ratio). (**C**) Immunohistochemical detection of KGFR. (**D**) Competition test on the serial section by adding an excess amount of 10-1 synthetic peptides to anti-KGFR IgG (500-fold at a molar ratio). (**E**) Normal rabbit IgG in place of anti-KGF IgG. (**F**) Normal rabbit IgG in place of anti-KGFR IgG. Immunohistochemical detection of KGF (**G**), AR (**H**) and Ki-67 (**I**). Arrows indicate positive cells. PG, Prostate gland; SS, Stroma surrounding prostate gland. Bar=50 μm.

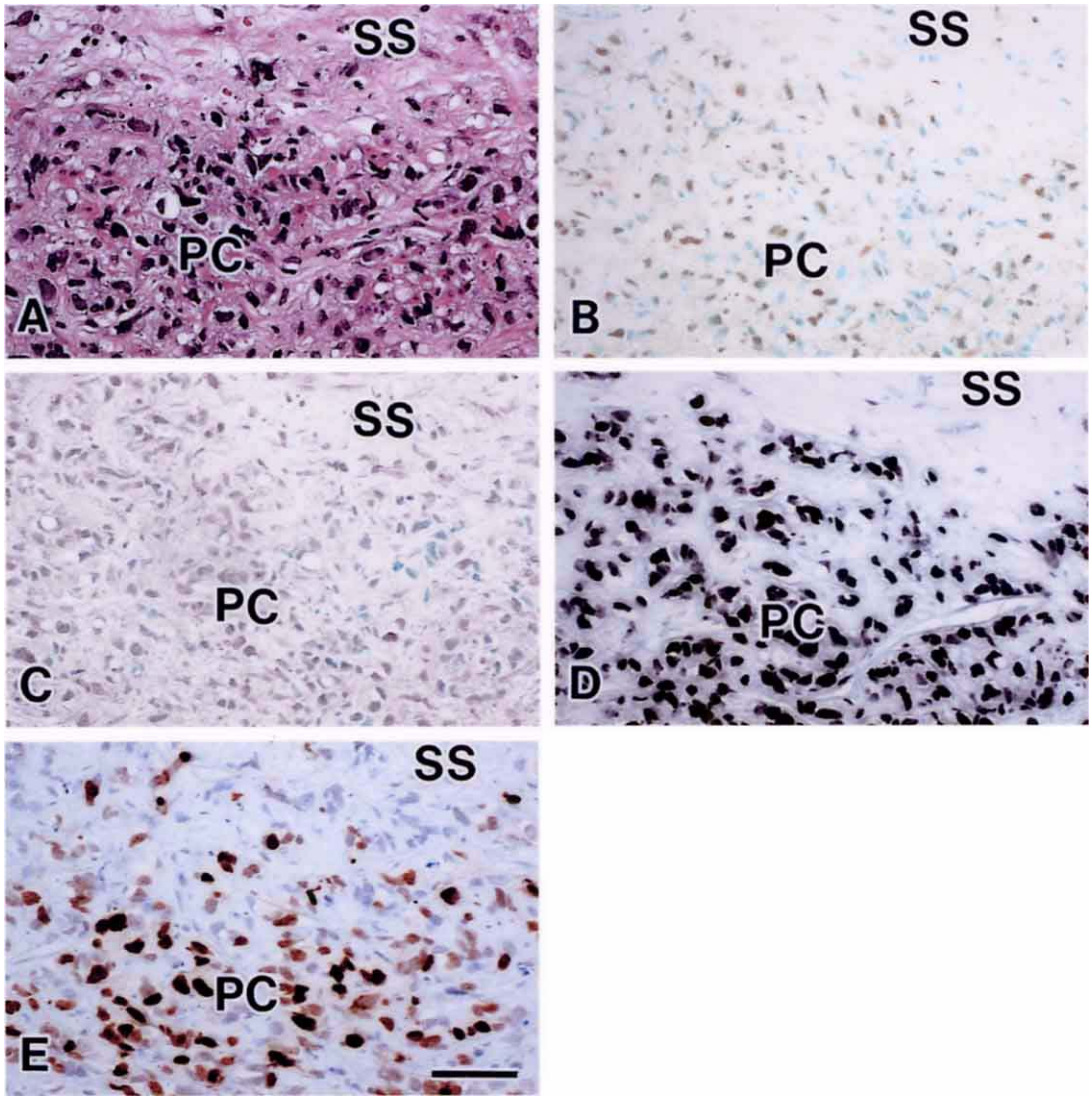

Fig. 3. Immunohistochemistry of KGF, KGFR, AR and Ki-67 in a representative prostate cancer of Gleason grade 5 in serial sections obtained from prostate needle biopsy specimens. (**A**) H & E staining. (**B**) KGF. (**C**) KGFR. (**D**) AR. (**E**) Ki-67. PC, Prostate cancer; SS. Stroma surrounding cancerous region. Bar=50 μm.

positive cells was expressed as a percentage of positive cells per total counted cells (Ki-67 LI; mean±SD).

Statistical analysis

Fisher's exact test was used to analyze association between KGF and/or KGFR expression and various prognostic parameters including age, serum prostate-specific antigen (PSA) levels, Gleason score, bone metastasis ratio, Ki-67 LI and stromal AR expression. Relapse-free survival curves were calculated according to the Kaplan-Meier method for evaluation of hormone-refractory condition and statistical significance was evaluated using the log rank test. A p-value less than 0.05 denoted the presence of significant difference. All analyses were performed using a statistical software package (StatView, ver. 4.5; Abacus Concepts, Berkeley, CA).

III. Results

Western blotting of KGF and KGFR

To assess the specificity of the novel antibodies for KGF and KGFR, we first performed western blotting of rh KGF and rh KGFR as authentic materials. Both rh KGF and rh KGFR were recognized by anti-KGF IgG and anti-KGFR IgG as bands with their predicted molecular weights of 19-kDa and 100-kDa, respectively (Fig. 1). Moreover, to confirm the expression of KGF and KGFR in human prostate cancers, the extracts from a typical specimen which was positive to both KGF and KGFR by immunohistochemistry were analyzed similarly. As shown in Figure 1, an intense band corresponding to 28-kDa or 135-kDa was detected with anti-KGF IgG and anti-KGFR IgG, respectively.

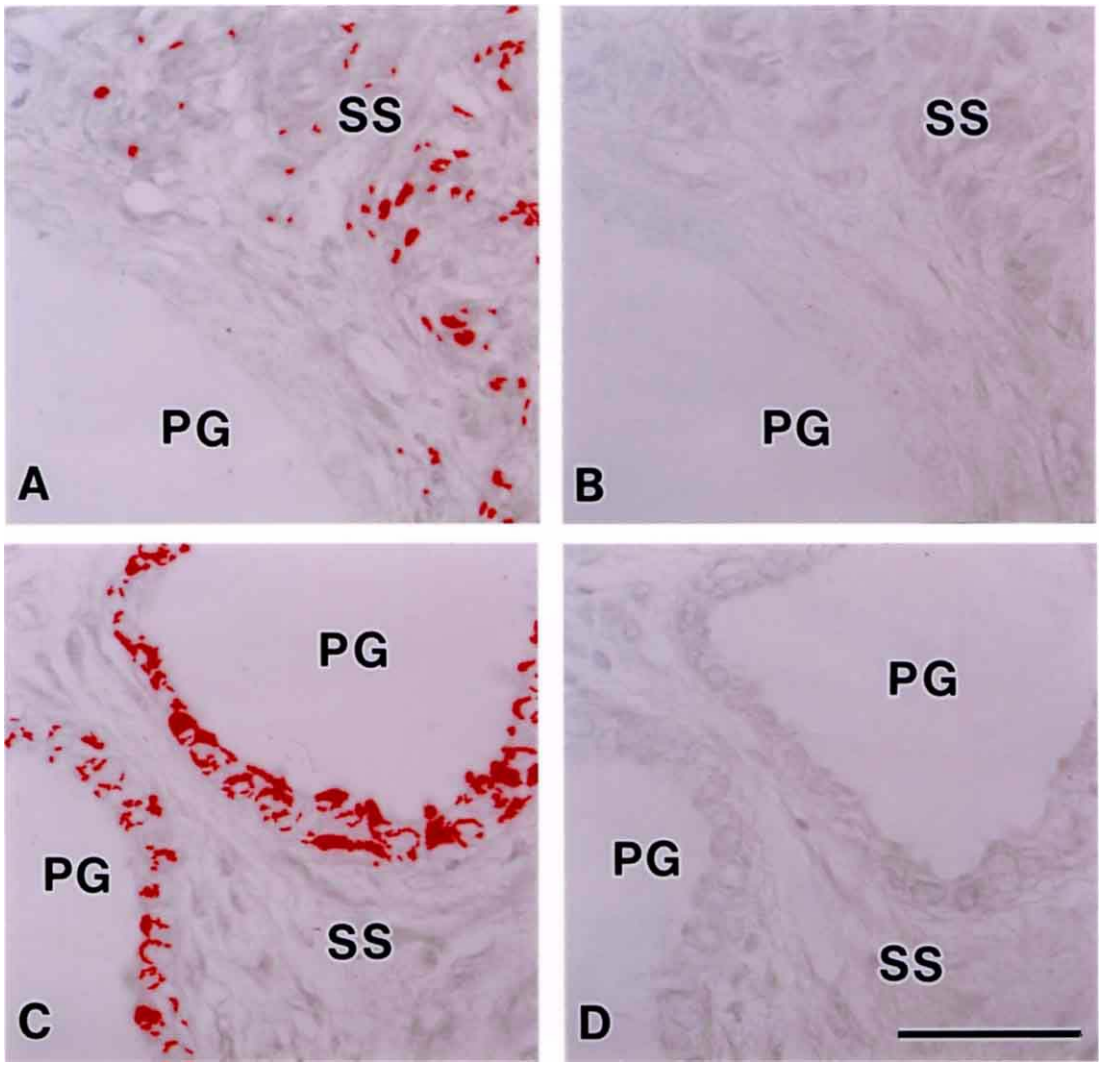

Fig. 4. *In situ* hybridization of KGF and KGFR mRNAs in normal prostate. Adjacent sections were hybridized with KGF antisense probe (**A**) or KGF sense probe (**B**) as a negative control. Similarly, sections were hybridized with KGFR antisense probe (**C**) or KGFR sense probe (**D**) as a negative control. The red color was assigned to positive signals by an image analyzer. PG, Prostate gland; SS, Stroma surrounding prostate gland. Bar=50 μm.

Localization of KGF and KGFR proteins

As shown in Figure 2, the signal for KGF in non-cancerous prostate tissue was detected in stromal cells as well as in glandular epithelial cells (Fig. 2A, G). In glandular epithelial cells, the signal for KGF was detected predominantly in basal epithelial cells, but not in secretory luminal cells. On the other hand, KGFR was localized in glandular epithelial cells including basal and secretory luminal cells as well as some endothelial cells in blood capillaries (Fig. 2C). To assess the specificity of these antibodies, we conducted peptide competition studies. When non-cancerous tissues were reacted with anti-KGF IgG in the presence of an excess amount (500-fold at a molar ratio) of the synthetic antigen oligopeptide, the staining of stromal and epithelial cells for KGF was markedly decreased (Fig. 2B). Similarly, the staining of glandular epithelial cells for KGFR was also decreased by the addition of an excess amount (500-fold at a molar ratio) of the corresponding synthetic oligopeptide to the primary antibody solution (Fig. 2D). When the primary antibody was replaced with normal rabbit IgG, no staining was observed (Fig. 2E, F).

As summarized in Table 3, in 14 cases of prostate cancer coexpression of KGF and KGFR was found and in 27 cases either KGF or KGFR alone was expressed. In the co-expressed cases, 7 cases expressed KGF and KGFR in foci of both primary and secondary Gleason grades, and other 7 cases showed coexpression of KGF and KGFR in foci of either primary or secondary Gleason grade. Very clearly, the coexpression of KGF and KGFR was significantly associated with the cancerous tissues with high Gleason scores 9 to 10, compared to the cases with Gleason scores 5 to 8 ($p=0.035$, Table 3). A typical example of prostate cancer with high Gleason grade, which expressed both KGF and KGFR, is shown in Figure 3 (B and C). In addition, the stain-

ing for KGF and KGFR in cancerous prostate tissues was almost abolished by the addition of an excess amount of the appropriate synthetic oligopeptide (data not shown).

Localization of KGF and KGFR mRNAs

To verify the synthesis of KGF and KGFR proteins, we performed nonradioactive *in situ* hybridization for KGF and KGFR mRNAs. Prior to the ISH experiments, the tissues were assessed for RNA integrity and its hybridizability using 28S rRNA staining. A strong staining for 28S rRNA in the cytoplasm as well as nucleoli was observed in 35 out of 41 cancerous specimens and 7 out of 9 non-cancerous prostate specimens (data not shown). We used these 42 specimens with appropriate preservation of 28S rRNA for a further investigation.

In non-cancerous prostate tissues, KGF mRNA was detected solely in stromal cells, but not in epithelial cells (Fig. 4A), in contrast to the immunohistochemical findings. On the other hand, when the sections were hybridized with KGFR antisense probe, KGFR mRNA was only observed in glandular epithelial cells (Fig. 4C), which was consistent with the results of immunohistochemistry. As a negative control, when adjacent sections were hybridized with KGF or KGFR sense probe, no staining was observed (Fig. 4B, D). In prostate cancer tissues with coexpression of KGF and KGFR proteins, both stainings of KGF and KGFR mRNA were detected in cancer cells (Fig. 5A, C). Again in control sections, no staining was observed (Fig. 5B, D). On the other hand, in prostate cancer tissues with either KGF or KGFR alone, KGF mRNA was expressed in the fibroblastic cells surrounding the cancerous region but not in cancer cells, while KGFR mRNA was localized in cancer cells (data not shown).

Relationship between KGF and AR expression

To examine whether the staining for KGF was correlated with AR expression, we conducted immunohistochemistry for KGF and AR in exactly adjacent sections. In non-cancerous prostate tissues, the signal for AR was detected in most of the stromal and glandular epithelial cell nuclei. Moreover, AR was colocalized with KGF in stromal cells but not in glandular epithelial cells (Fig. 2G, H). On the

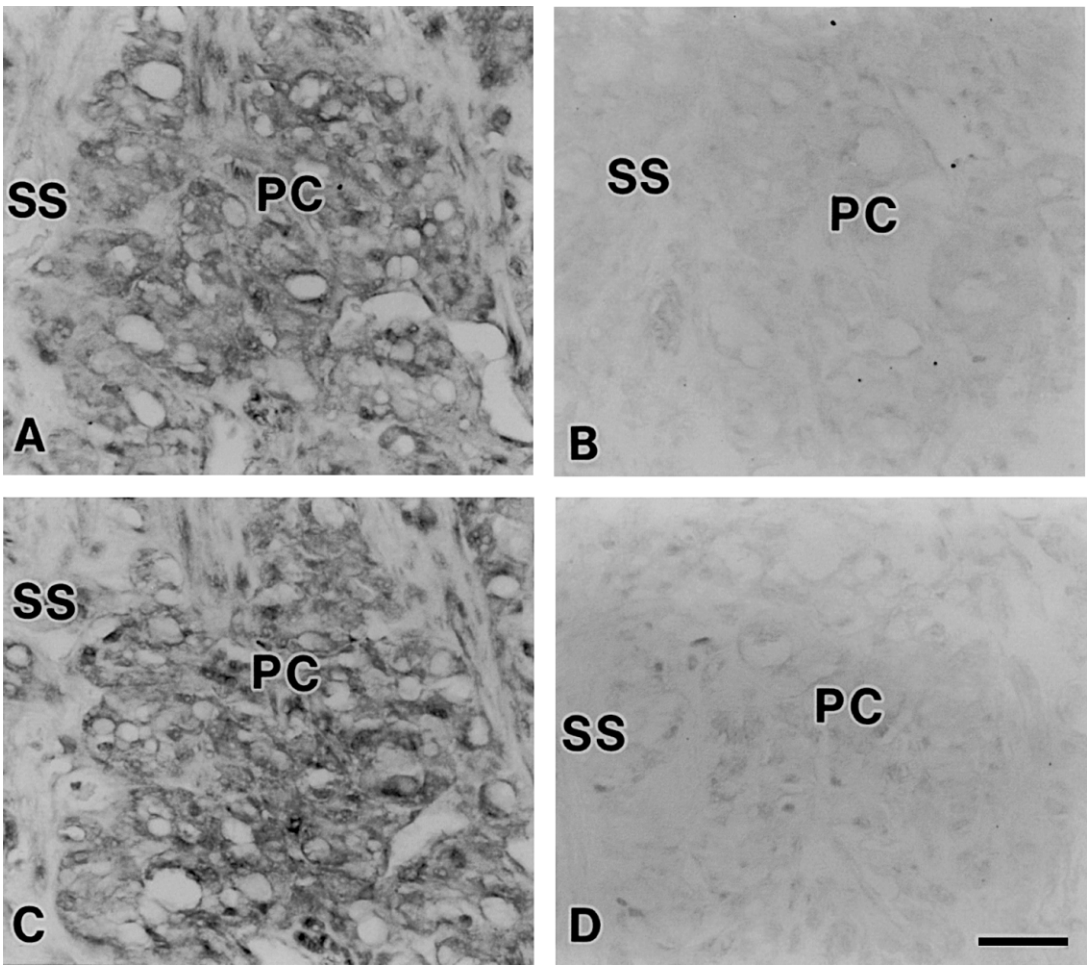

Fig. 5. *In situ* hybridization of KGF and KGFR mRNAs in cancerous prostate tissue of Gleason grade 4. Adjacent sections were hybridized with KGF antisense probe (**A**) or KGF sense probe (**B**) as a negative control. Similarly, sections were hybridized with KGFR antisense probe (**C**) or KGFR sense probe (**D**) as a negative control. PC, Prostate cancer; SS, Stroma surrounding cancerous region. Bar=50 μm.

other hand, in cancerous prostate tissues, AR expression in stromal cells surrounding cancerous regions seems to be decreased in tissues with increasing Gleason grades (Fig. 3D). Irrespective of Gleason grade, AR was expressed strongly in the majority of cancer cells.

Cell proliferation assessed by Ki-67 immunostaining and its relation with the expression of KGF and KGFR

We also conducted immunohistochemistry of Ki-67 in the serial sections to address whether the staining for KGF and KGFR was associated with proliferating activity. In non-cancerous prostate specimens, Ki-67 staining was noted exclusively in the basal layer of glandular epithelial cells. Furthermore, some, but not all, of KGF-positive epithelial cells were also positive for Ki-67 (Fig. 2G, I). In cancerous prostate tissues, Ki-67 LI was significantly higher in the cases coexpressing KGF and KGFR (Fig. 3E), compared to the cases with either KGF or KGFR alone ($p<0.001$, Table 3).

Correlation between bone metastasis ratio and expression of KGF and/or KGFR

Bone metastasis was observed 19 cases out of 22 prostate cancer patients with stage D. Bone metastasis ratio was significantly higher in patients with cancers coexpressing KGF and KGFR, compared to that expressing either KGF or KGFR alone ($p=0.026$, Table 3). However, other metastasis regions including lymph node were not significantly correlated with the coexpression of KGF and KGFR.

Correlation between hormone-refractory ratio and expression of KGF and/or KGFR

To examine the involvement of coexpression of KGF and KGFR in the development of hormone-refractory prostate cancer, we evaluated for relapse-free survival ratio. The relapse-free survival period of patients with cancers coexpressing KGF and KGFR was significantly shorter than that of patients with cancers expressing either KGF or KGFR alone (Fig. 6, $p<0.001$).

IV. Discussion

In the present study, we investigated the expression of KGF and KGFR in human diseased prostate tissues by *in situ* hybridization and immunohistochemistry to analyze the possible correlation between their expression and various prognostic parameters. The major findings of this study were as follows; in non-cancerous prostate tissues, both KGF mRNA and protein were exclusively localized in stromal cells, while those of KGFR were expressed in glandular epithelial cells. On the other hand, in the cancerous tissues, coexpression of KGF and KGFR at the level of mRNA and protein was significantly associated with the cancers of high Gleason scores. Moreover, prostate cancers with KGF and KGFR coexpression significantly exhibited a higher proliferative activity and a higher rate of bone metastasis, compared to that expressing either KGF or KGFR alone. The relapse-free survival period of patients with the cancers coexpressing KGF and KGFR was significantly shorter than that of the other patients. Thus, these results indicate that the androgen-independent autocrine and/or paracrine KGF-KGFR loop may be at least partly involved in the enhanced proliferative activity, bone metastasis and hormone insensitivity of prostate cancer cells with high Gleason scores.

McGarvey and Stearns [23] reported a strong mRNA expression of both KGF and KGFR in high Gleason grade

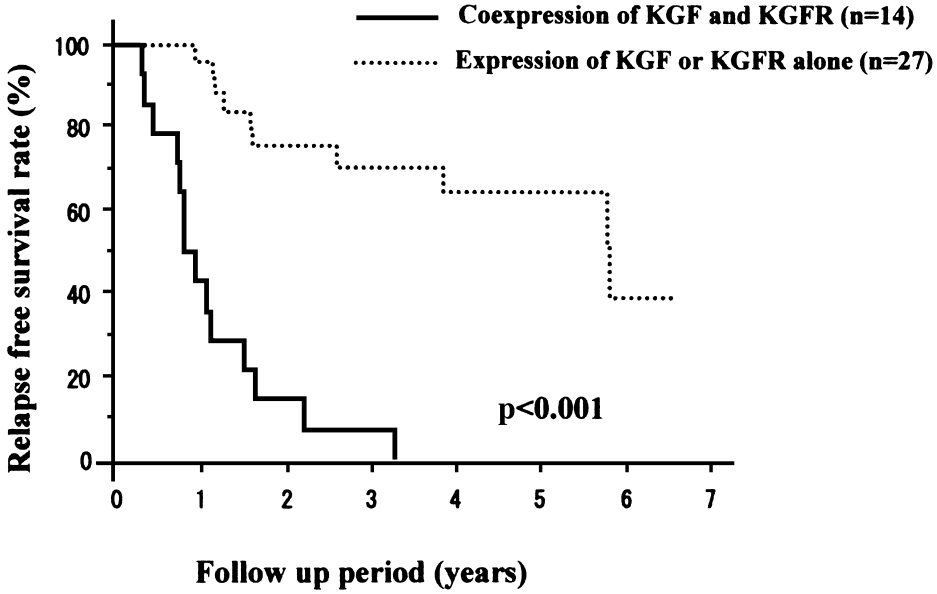

Fig. 6. Correlation between hormone-refractory ratio and expression of KGF and/or KGFR by Kaplan-Meier method. The relapse survival period of patients with tumors that coexpressed KGF and KGFR was significantly shorter than that of patients with tumors that expressed either KGF or KGFR alone. $p<0.001$.

cancer cells. We also confirmed the strong expression of KGF and KGFR proteins in the cancer cells. Moreover, we found that the coexpression of KGF and KGFR was significantly correlated with higher Ki-67 LI than that of the other cancers, indicating that KGF may play a mitogenic role in the prostate cancer, although it is now widely accepted that KGF has dual roles in the morphogenesis of normal lung and mammary gland through the regulation of differentiation as well as proliferation [9, 31].

The previous *in vitro* studies with primary cultures of human prostate tissues revealed that stromal cells, but not epithelial cells, expressed KGF mRNA, as analyzed by northern blotting and RT-PCR [12]. Similarly, in our study on normal and benign hyperplastic prostate tissues, the expression of KGF mRNA and protein was solely found in stromal cells, and seemed to be tightly associated with AR expression. Since the promoter of KGF gene is known to be activated by androgen-bound AR [4], production of KGF in stromal cells may depend on the presence of AR. Interestingly, however, we found that some glandular epithelial cells were positive to KGF protein, but not to its mRNA. This result is apparently inconsistent with the previous findings [23, 30], which reported the presence of both KGF mRNA and protein in glandular epithelial cells. Although the reason for this discrepancy is not clear at present, we should be aware of the possibility that the epithelial KGF can reflect the incorporation of KGF through KGFR. In this context, it should be also noted that AR, essential for the KGF mRNA expression, is not colocalized with KGF in glandular epithelial cells.

Recently, caveolin-1 was identified as one of the KGF-regulated genes [6]. Since caveolin-1 is known as a metastasis-related gene [41] as well as a target gene responsible for hormone-resistance of prostate cancer [26], it is reasonable to assume that KGF may also play an important role in the induction of distant metastasis and transition from androgen-sensitive to androgen-insensitive growth. In fact, in the present study, bone metastasis ratio was significantly higher in patients with the cancer coexpressing KGF and KGFR. Moreover, the relapse-free survival period of patients with the double positive cancer was significantly shorter than that with cancers expressing either KGF or KGFR alone. In addition, Leung *et al.* [21] reported that the expression of both KGF and KGFR mRNAs was upregulated in hormone-insensitive prostate cancers. These findings emphasize the need for further studies to investigate the involvement of co-expression of KGF and KGFR in the autonomous autocrine and/or paracrine loop and androgen insensitivity in prostate cancer.

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VI. References

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