Alterations in Expression of Basic Fibroblast Growth Factor (FGF) 2 and Its Receptor FGFR-1 in Human Prostate Cancer

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ABSTRACT
Fibroblast growth factors (FGFs) play an important role in the growth and maintenance of the normal prostate. There is increasing evidence from both animal models and analysis of human prostate cancer cell lines that alterations of FGFs and/or FGF receptors (FGFRs) may play an important role in prostate cancer progression. To better define the role of FGF2 and FGF7 in human prostate cancer in vivo, we have quantified these two growth factors in clinically localized human prostate cancers and uninvolved prostate by ELISA and Western blotting and determined their localization by immunohistochemistry. The expression of two of the primary receptors for these growth factors, FGFR-1 and FGFR-2, were also analyzed by immunohistochemistry and Western blotting in these same samples. We have found that FGF2 is significantly increased in prostate cancers when compared with uninvolved prostate and that the FGF2 is present in the stromal fibroblasts and endothelial cells but not the cancer cells. In addition, we have observed overexpression of both FGFR-1 and FGFR-2 in the prostate cancer epithelial cells in a subset of prostate cancers and that such overexpression is correlated with poor differentiation. Thus, there is both an increase in FGF2 concentration in prostate cancers and an increased expression of a receptor capable of responding to this growth factor, establishing a potential paracrine stimulation of prostate cancer cells by the surrounding stromal cells, which may play an important role in prostate cancer progression.

INTRODUCTION
Prostate cancer is the most common cancer in men in the United States and the second leading cause of cancer mortality in this group. Considerable progress has been made in recent years in understanding the molecular alterations in human prostate cancer, but significant gaps remain in our knowledge of the pathogenesis of this disease. Altered expression of growth factors and their receptors may play a key role in the establishment and progression in prostate cancer, acting by either autocrine or paracrine mechanisms to stimulate cell proliferation and/or prevent apoptosis, enhance invasion, and promote angiogenesis.

FGFs play an important role in the growth and maintenance of the normal prostate. The FGF gene family consists of 18 different genes encoding related polypeptide mitogens. It has been shown that some FGFs are mitogenic for prostatic epithelial cells in culture (1) and are produced by stromal cells in the normal prostate, consistent with a paracrine stimulation of epithelial growth (2). FGFs bind to a family of four distinct, high-affinity tyrosine kinase receptors, designated FGFR-1–4 (for review, see Ref. (3)). These receptors consist of an extracellular portion containing 3 immunoglobulin-like domains and an intracellular tyrosine kinase domain. FGFRs 1–3 all undergo an alternative splicing event in which two alternative exons (IIb and IIc) can be used to encode the COOH-terminal portion of the third immunoglobulin-like loop, which results in receptor isoforms with dramatically altered binding specificity for different FGFs. We have shown previously (2) that normal prostatic epithelial cells in culture express the FGF7 binding (IIIB) isoform of FGFR-2 as well as the FGFR-3 IIc isoform but do not express FGFR-1 or FGFR-4.

The role of FGFs and FGFRs in prostate cancer is still unclear. Expression of FGF2 has been demonstrated in the DU145 and PC3 prostate cancer cell lines (4), and Greene et al. (5) have shown that such expression of FGF2 was increased in highly metastatic sublines of the PC3 cell line when compared with less metastatic sublines. Overexpression of FGF7 (6) has been detected by in situ hybridization. Ropiquet et al. (7) have studied the effects of expression of FGF2 under the control of a strong promoter on immortalized but nontumorigenic human prostatic epithelial cells. They found that these cells had an increased proliferation rate and had acquired the ability to form colonies in soft agar, although they were still not tumorigenic in nude mice. A recent report has shown that, particularly in the presence of exogenous FGF2, that PC3 prostate cancer cell lines interact with stromal cells to promote angiogenesis in a culture system (8). In summary, there is evidence that FGFs may be overexpressed in human prostate cancer, and such overexpression may alter the properties of the prostatic epithelial cells to increase tumor proliferation, angiogenesis, and possibly metastasis.

To better define the role of FGF2 and FGF7 in human prostate cancer.

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3 The abbreviations used are: FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; DAB, diaminobenzidine; IHC, immunohistochemistry.
prostate cancer, we have quantitatively analyzed the expression of these two growth factors in human prostate cancers and normal prostate by ELISA and Western blotting and determined their localization by immunohistochemistry. In addition, we have analyzed the expression of two of the primary receptors for these growth factors, FGFR-1 and FGFR-2, by immunohistochemistry and Western blotting in these same samples. We have found that FGF2 is significantly increased in prostate cancers relative to normal prostate and that the FGF2 is expressed in the peritumoral stromal cells, consistent with a role for FGF2 as a paracrine growth factor for prostate cancer cells. Furthermore, we have observed overexpression of both FGFR-1 and FGFR-2 in a subset of prostate cancers and that such overexpression correlates with poor differentiation. Thus, there is both an increase in FGF2 concentration in prostate cancers and an increased expression of a receptor capable of responding to this growth factor, establishing a potential paracrine stimulation of prostate cancer cells by the surrounding stromal cells, which may play an important role in prostate cancer progression.

MATERIALS AND METHODS

Tissue Acquisition and Pathological Analysis. All prostate cancer tissues and samples of the uninvolved peripheral zone of the prostate were taken from radical prostatectomies performed for treatment of clinically localized, Stage B prostate cancer. Tissues were received fresh and portions snap frozen in liquid nitrogen or used to establish primary cell cultures (see below). In each case, the tissue remaining after harvest of fresh tissue, including the entire prostatic capsule and both seminal vesicles, were subject to full pathological analysis of tumor stage (American Joint Committee on Cancer) and grade (Gleason score). The frozen tissues were then analyzed by frozen section to confirm the presence or absence of carcinoma and, if present, the percentage of the tissue involved by cancer. The carcinoma tissues contained 20–90% cancer (average 56%) whereas all of the normal peripheral zone tissues were free of cancer. Additional frozen sections were used to prepare immunohistochemistry, and the remaining tissue was used to prepare cell extracts.

Growth Assays. Primary epithelial cell cultures were established using prostatic tissue samples from areas in radical prostatectomy specimens that were not involved by carcinoma. Primary epithelial cells were plated on collagen coated 35-mm dishes at $10^4$ cells per dish in complete epithelial growth medium, which includes bovine pituitary extract (a source of FGFs), epidermal growth factor, insulin, dexamethasone, cholera toxin, and BSA as described previously (2). We have previously shown such cultures to be of prostatic epithelial origin by immunohistochemistry with antibodies to prostate specific antigen and cytokeratin (2). The next day, the medium was changed to epithelial growth medium without bovine pituitary extract. Cells were kept in this incomplete medium as controls or were supplemented with either 1, 10, or 100 ng/ml recombinant FGF2 (R&D Systems, Minneapolis, MN). Cells were then trypsinized and counted using a Coulter counter after 24 h and then at 2-day intervals.

Preparation of Cell Extracts. Prostatic tissue samples were weighed quickly, pulverized in liquid nitrogen, and then homogenized by 3 strokes, each for 10 s, on ice, in a lysis buffer containing 20 mM HEPES (pH 7.4), 2 mM EDTA, 250 mM NaCl, 0.1% NP40, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 0.5 mg/ml benzamidine, and 1 mM phenylmethylsulfonyl fluoride (PMSF) using 0.5 ml lysis buffer per 200 mg of tissue. The homogenate was then incubated for 30 min on ice, and insoluble material was removed by centrifugation for 1 min in a microcentrifuge at 4°C. The protein content of the supernatant was determined by the method of Bradford (9). These extracts were then used directly for ELISA or Western blotting to detect FGFs or for heparin-affinity purification before Western blotting to detect FGFs.

Heparin-Affinity Purification and Western Blot Analysis. For heparin-affinity purification of FGFs, 150–300 μg of protein extract from eight individual prostate cancer extracts were incubated with 50 μl of heparin agarose overnight at 4°C with agitation. The beads were then washed in buffer containing 10 mM HEPES (pH 7.4), 25 mM NaCl, and 1 mM DTT. The washed beads were then boiled in sample buffer and centrifuged, and the supernatant was subjected to SDS-PAGE using a 15% gel. The resolved proteins were electrotransferred to nitrocellulose membranes and then blocked with PBS with 0.5% Tween 20 and 5% fat-free milk. The membrane was then incubated with either 400 ng/ml goat polyclonal anti-FGF7 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or 100 ng/ml rabbit polyclonal anti-FGF2 antibody (Santa Cruz Biotechnology) at 4°C. After overnight incubation, membranes were washed with PBS with 0.5% Tween 20 and treated with an appropriate secondary antibody conjugated to horseradish peroxidase at a concentration of 80 ng/ml (Santa Cruz Biotechnology). The antigen-antibody reaction was visualized using an enhanced chemiluminescence assay (Amersham, Arlington Heights, IL) and exposure to enhanced chemiluminescence film (Amersham). Bands were quantitated using a Molecular Dynamics densitometer.

For detection of FGFRs, 50 μg of protein from each of six prostate cancer extracts were loaded per lane, and Western blotting was performed as described above, using 500 ng/ml rabbit polyclonal anti-FGFR-1 antibody (Santa Cruz Biotechnology) or rabbit polyclonal anti-FGFR-2 antibody (Santa Cruz Biotechnology) at 500 ng/ml.

Immunohistochemistry. Frozen tissue sections were fixed in acetone for 10 min, postfixed in methanol for an additional minute, and stored at −80°C. Immunohistochemical localization of FGF2 or FGF7 was carried out by the avidin-biotin complex method as described earlier (10). All of the sections were treated with Autoblocker (R&D Systems) to inhibit endogenous peroxidase and avidin/biotin (Vector Laboratories, Burlingame, CA) to block endogenous biotin. The sections were incubated with rabbit polyclonal anti-FGF2 antibody (R&D Systems) at 200 ng/ml, mouse monoclonal anti-FGF2 antibody (Oncogene Research Products, Cambridge, MA) at 100 ng/ml, or goat polyclonal anti-FGF7 antibody (Santa Cruz Biotechnology) at 400 ng/ml, all at 4°C for 12 h. A total of 31 prostate cancers and 6 sections from uninvolved peripheral zone were analyzed. After liberal washing with PBS (pH 7.4), sections were then incubated with appropriate biotinylated secondary antibody at 1:200 dilution (Vector Laboratories). Sections were then washed with PBS containing 0.1% Tween 20 and incubated with avidin-biotin complex (Vectorstain Elite,
Vector Laboratories) for 15 min. The antigen-antibody reaction was demonstrated using DAB as substrate, and the sections were then counterstained with hematoxylin. The specificity of staining was evaluated by preincubation of antibody with excess recombinant FGF2 or FGF7. This pretreatment completely abolished immunostaining for the corresponding antibody.

Immunostaining for FGFRs was carried out on 27 prostate cancers and 6 uninvolved peripheral zone tissues using the protocol described above using either anti-FGFR-1 (Santa Cruz Biotech) or anti-FGFR-2 (Santa Cruz Biotech), both at 1 μg/ml. The antigen-antibody reaction was demonstrated using VIP (Vector) or DAB as substrate. Preincubation of the antibodies with the corresponding immunizing peptide completely abolished immunostaining for that antibody.

ELISA. The FGF2 concentration in tissue extracts was measured in the extracts of 31 cancers and 11 normal peripheral zone tissues by ELISA, which is based on a quantitative sandwich immunoassay technique. Assays were performed according to the manufacturer’s instructions in a 96-well plate using 4 μl of tissue extract, prepared as described above, per well. FGF7 concentration was determined in extracts of 26 prostate cancers and 8 uninvolved peripheral zone tissues using a human FGF7 ELISA (R&D Systems) and 30 μl of tissue extract.

RESULTS

Concentration of FGF2 and FGF7 in Human Prostate Cancer Tissue. To determine whether FGF2 and/or FGF7 expression is increased in human prostate cancer, we directly analyzed tissue extracts from prostate cancers and uninvolved prostatic peripheral zone tissue by ELISA. For FGF2, a total of 31 cancer and 11 normal peripheral zone extracts were analyzed. Results are shown in Fig. 1. The tissue concentration of FGF2 was quite variable, particularly for the prostate cancers. However, two-thirds of the cancers had higher FGF2 concentrations than the highest uninvolved tissue, and some cases had extremely high levels of FGF2 (>400 ng/gm tissue). The mean concentration of FGF2 in the prostate cancers was markedly increased relative to control tissue, with a mean FGF2 concentration of 271 ng/g wet weight of tissue versus 110 ng/g wet weight for uninvolved peripheral zone. This difference is statistically significant (P < 0.005, t test). There was no correlation of FGF2 content with Gleason score or pathological stage.

We also analyzed a total of 26 prostate cancers and 8 uninvolved peripheral zone samples for FGF7 content. The mean FGF7 content in the prostate cancers was 36.1 ng/g wet weight tissue, and uninvolved peripheral zone had a mean FGF7 content of 28 ng/g wet weight. This difference was not statistically significant (P = 0.41, t test).

To confirm the results of the ELISA, we analyzed a subset of prostate cancer extracts by Western blotting with either anti-FGF2 or anti-FGF7 antibodies after partial purification of heparin-binding proteins from the cell extracts using heparin-agarose. Using anti-FGF2 antibodies, we detected a band of variable intensity at 18 kDa, consistent with the known size of FGF2 translated from the AUG codon (11), in all of the extracts (Fig 2A). Densitometric analysis of this band and comparison of
the absorbance to the FGF2 concentration as determined by ELISA show an excellent correlation between these two determinations (Fig. 2B). Similar experiments using anti-FGF7 antibodies likewise confirmed the specificity and accuracy of the ELISA (data not shown).

**Immunohistochemistry with Anti-FGF2 and Anti-FGF7 Antibodies.** Having established the marked increase in FGF2 concentration in prostate cancers, we wished to determine its localization within the prostate cancer tissue by immunohistochemistry. In normal prostate, anti-FGF2 antibodies, both monoclonal and polyclonal, showed immunoreactive FGF2 in stromal cells with fibroblastic morphology and in endothelial cells. No staining of epithelial cells was seen. These results are consistent with our prior analysis of cultured prostatic stromal and epithelial cells that showed strong expression in stromal cells and expression detectable only by reverse transcription-PCR in epithelial cells (2). Immunohistochemistry of frozen sections from the 31 cancers used for determination of FGF2 concentration showed the same pattern of staining using either antibody, with no evidence of staining of the neoplastic epithelial cells (Fig. 3), not even in those with extremely high FGF2 concentration. No variation in the staining intensity of individual cells was noted, but the number of positively staining fibroblastic and endothelial cells was quite variable, both in different regions of the same sample and among different tissue samples. In the majority of cases, there was a marked increase in the number of positively staining cells per unit area of stroma between, and adjacent to, the neoplastic epithelium, although some cases did not show such an increase. Immunohistochemistry using anti-FGF7 polyclonal antibody showed strong staining of stromal fibroblastic cells, but again no staining was seen in prostate cancer epithelial cells (data not shown). The distribution of the positively staining cells was similar to that seen using anti-FGF2 antibodies.

**FGF2-induced Proliferation of Normal Prostatic Epithelial Cells.** Given the high level of FGF2 in the prostate cancer tissues, we wished to determine whether it might act as a paracrine stimulator of growth for the transformed prostatic epithelial cells. The effect of FGF2 on the proliferation of normal prostatic epithelial cells reported by other laboratories has been variable (1, 12). We, therefore, tested the proliferative response of prostatic epithelial cells from tissue uninvolved by cancer to exogenous FGF2. As can be seen in Fig. 4, there is a statistically significant mitogenic response to as little as 1 ng/ml of exogenous FGF2 after 5 days in culture, with an even more pronounced effect at 10 and 100 ng/ml. Therefore, FGF2 is mitogenic for prostatic epithelial cells at concentrations corresponding to less than one percent of its level in prostate cancer tissue.

**Expression of FGFR-1 and FGFR-2 in Prostate Cancer.** Because we had observed both FGF2 and FGF7 in prostate cancer tissues, it was pertinent to determine whether two of the primary receptors for these growth factors were present on the prostate cancer cells *in vivo*. We, therefore, analyzed 6 normal peripheral zone tissues and 27 prostate cancers for expression of FGFR-1 and FGFR-2 by immunohistochemistry using frozen sections corresponding to the tissues analyzed for FGF2 and FGF7 expression above. Immunohistochemistry of uninvolved peripheral zone tissue with anti-FGFR-1 antibody showed staining of stromal cells and endothelial cells of blood vessels. Basal epithelial cells stained strongly for FGFR-1, but luminal epithelium was negative (Fig. 5A). These findings are in accordance with...
with those reported by Hamaguchi et al. (13) using a different anti-FGFR-1 antibody. Immunohistochemistry with anti-FGFR-2 antibody also showed strong staining of endothelial cells but not stromal cells, consistent with our prior analysis of cultured stromal cells. The basal epithelial cells were again stained strongly, but luminal epithelial cells were negative. Analysis of the prostate cancers with anti-FGFR-1 antibodies revealed positive immunostaining in approximately 20% of cases. An example of such staining is shown in Fig. 5B. Positive immunostaining with anti-FGFR-2 antibodies was slightly more common, occurring in 30% of cases. As can be seen in Fig. 6, expression of both FGFR-1 and FGFR-2 was more common in poorly differentiated (Gleason 8–9) cancers. The difference in the rate of FGFR-1 expression between well-differentiated (Gleason 4) and either moderately (Gleason 5–7) or poorly differentiated (Gleason 8–9) cancers was statistically significant by \( \chi^2 \) analysis (\( P \leq 0.001 \)). Although there was a trend for higher rates of expression of FGFR-2 in more poorly differentiated tumors, these differences were not statistically significant.

We sought to confirm our immunohistochemistry results by analyzing a small subset of our prostate cancers by Western blotting with anti-FGFR-1 and FGFR-2 antibodies. These experiments were complicated by the normal basal expression of both of these receptors in a fraction of epithelial and stromal cells, the variable percentage of normal epithelium and stroma in the tissue samples as well as the variable percentage of tumor in each specimen, but we were able to confirm the expression of FGFR-1 and FGFR-2 by the cancer cells in some cases. Fig. 7 shows the results of these studies. Western blotting with anti-FGFR-2 antibodies gave a single band in all of the samples of approximately 125 kDa that was more intense than the FGFR-2 band, consistent with its expression in stroma and the lack of FGFR-2 expression by stromal cells. The variability in staining in Lanes 1–4 reflects the variability in stromal content. Given this more intense but variable staining due to stronger FGFR-1 stromal expression, we were not able to distinguish an increase in band intensity in case 6, which contains 50% carcinoma. However, case 5, as mentioned above, is almost pure tumor with very little stroma; therefore, the presence of a band for FGFR-1 in this sample confirms the positive immunostaining for FGFR-1 inasmuch as the FGFR-1 band should be markedly reduced in this sample because of the absence of normal prostatic stromal tissue if no expression of FGFR-1 were present in the cancer cells. Thus, although Western blotting is not completely satisfactory for the analysis of FGFR expression in human prostate cancer samples because of inherent difficulties in sample purity, it was possible to confirm our IHC results in some cases. Expression of FGFR-2 in tumor cells was easier to detect than FGFR-1 because of the lower basal expression; therefore, the presence of FGFR-2 expressing tumor increased the FGFR-2 content of the tissue sample substantially, whereas FGFR-1 expressing tumor would in large part displace tissues that already expressed substantial quantities of FGFR-1, and, therefore, there would be little change in the total tissue content of FGFR-1.

Fig. 4  Proliferative response of primary prostatic epithelial cells to FGF2. Primary cultures of prostatic epithelial cells were established in epithelial growth media as described in “Materials and Methods” and plated at 5 \( \times \) 10^4 cells per 35-mm dish. Cells were then placed in epithelial growth medium without bovine pituitary extract and incubated in this medium alone or with the addition of 1, 10, or 100 ng/ml of recombinant FGF2. Cells were refed at 48 h intervals, and cell number was determined at the indicated intervals by counting using a Coulter counter. All of the determinations were in triplicate. Bars, SDs of the triplicate determinations.
DISCUSSION

In this report, we have shown that the FGF2 content of prostate cancer tissues is significantly increased in comparison with normal prostate tissue. Immunohistochemistry with anti-FGF2 antibodies indicates that this increased FGF2 is localized to the stroma of the cancers. Sinowatz et al. (14) have also reported immunolocalization of FGF2 to the stroma of prostatic carcinomas, with absent or very weak staining of the neoplastic epithelium, similar to our finding. FGF2 is poorly secreted because of its lack of signal sequence (15); therefore, the majority of the FGF2 is retained within the cell. The presence of FGF2 within stromal fibroblasts and endothelial cells thus indicates that it is almost certainly synthesized by these cells in vivo. Yamazaki et al. (16) have examined pancreatic carcinomas by both in situ hybridization and immunohistochemistry and found that the FGF2 mRNA is expressed in cells containing FGF2 identifiable by immunohistochemistry. The fact that two different antibodies, one monoclonal and one polyclonal, gave identical results, strengthens our conclusion that the increased FGF2 is present in the stromal cells but not in the prostate cancer cells. It should be noted that all of the prostate cancer tissue sections contain internal positive controls for FGF2 immunostaining, i.e., the stromal fibroblasts and endothelial cells. Our laboratory (2) and others (12) have shown that cultured prostatic stromal cells express FGF2, which is consistent with the prominent staining of these cells seen in vivo by immunohistochemistry. Other investigators have noted staining of blood vessels with anti-FGF2 antibodies in a variety of tissues (17–19), similar to what we observed in the prostate. Therefore, we are confident that the increased FGF2 content in the cancers is due to induction of stromal FGF2 production. The mechanism of such FGF2 induction is unclear but clearly merits further investigation. This is in contrast to the increased FGF2 production by the cancer epithelial cells themselves observed by immunohis-

**Fig. 5** Immunohistochemistry with anti-FGFR-1 antibodies. Uninvolved peripheral zone or prostate cancers were stained with anti-FGFR-1 antibodies as described in “Materials and Methods.” Antigen-antibody complexes were visualized with DAB substrate followed by counterstaining with hematoxylin. A, uninvolved peripheral zone. Dark arrows, staining of basal cells. Luminal epithelial cells are negative (open arrow). ×400. B, prostate cancer. Shown are poorly differentiated neoplastic cells that have variable staining with anti-FGFR-1 antibodies. Dark arrows, Some of the more intensely stained cells. ×400.
tochemistry in a number of other carcinomas including pancreatic (16), breast (18, 20), hepatocellular (21), non-small cell lung (22), and head and neck squamous (17) carcinomas. It should be noted that increased production of FGF2 by cancer cells themselves may occur in more advanced prostate cancers because it is well known that a number of prostate cancer cell lines (such as DU145 and PC3) that were established from metastatic cancers express FGF2 (4).

There are a number of potential biological effects of the increased FGF2 present in the prostate cancers. In this report, we show that FGF2 is mitogenic for normal prostatic epithelial cells in culture, although it is less effective than FGF7 in this regard. The mitogenic effect of FGF2 in normal prostatic epithelial cells is probably mediated by FGFR-3, which we have previously shown to be strongly expressed by prostatic epithelial cells (2), because FGFR-1 is not expressed in normal prostatic epithelial cells in culture (2). Studies by Ornitz et al. (23) have shown that FGF2 binding to FGFR-3 can lead to a potent mitogenic response. Supporting our observation, Ropiquet et al. (7) have shown that immortalized but nontumorigenic prostatic epithelial cells have enhanced proliferation after stable transfection of an FGF2 expression construct. It is known that FGF2 can induce production of matrix metalloproteinases (20, 24), which could enhance tissue invasion by the tumor cells in vivo. Finally, FGF2 is well known as an angiogenic factor and can potentially lead to enhanced tumor angiogenesis (15). Thus, the induction of FGF2 production by peritumoral stromal cells could lead to enhanced proliferation, invasion, and angiogenesis in prostate cancer.

We did not find an increased content of FGF7 in clinically localized prostate cancers and found no evidence of FGF7 protein in the cancer cells by immunohistochemistry. This is in contrast to prior reports of increased FGF7 mRNA in prostate cancer cells by in situ hybridization (6). However, FGF7 is a potent growth factor for prostatic epithelial cells and is present in biologically significant concentrations in the cancer tissues; therefore, it could contribute to the growth of the cancer cells in vivo depending on the isoform of FGFR-2 expressed (see below).

In addition to overexpression of FGF2, we have shown that there is increased expression of both FGFR-1 and FGFR-2 in poorly differentiated prostate carcinomas. In well-differentiated prostate cancers, there is no detectable expression of FGFR-1, similar to prostatic luminal epithelial cells and to primary epithelial cells in culture. There is detectable expression of FGFR-1 in 18% of moderately differentiated and in 40% of poorly differentiated cancers. Unfortunately, antibodies specific for all of the isoforms of FGFR-1 are not available; thus, we do not know the isoform of the FGFR-1 being expressed in these cells. However, the data of Ornitz et al. (23) indicate that both FGFR-1 IIIc and FGFR-1 IIIb respond mitogenically to FGF2. Thus, increased expression of either isoform could increase the sensitivity of the cancer cells to FGF2 produced in the surrounding stroma. It should be noted that a similar induction of expression of FGFR-1 is observed in both the DU145 and PC3 prostate cancer cell lines (25), and that these cell lines both have induction of expression of FGF2 to much higher levels than normal prostatic epithelial cells, establishing an autocrine loop in these cell lines derived from metastatic cancers. Establishment of similar autocrine loops has been reported in the Dunning rat model system. Yan et al. (26) have shown that as these transplantable tumors progress from a mixed stromal-epithelial phenotype to a stromal independent phenotype the expression of FGFs not originally present in the tumors occurs, and that there are changes in the isoforms of FGFRs expressed, consistent with autocrine stimulation of growth. Thus the cancer becomes independent of the stromal paracrine factors by the establishment of autocrine FGF stimulation that could promote both invasion

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4 Unpublished observations.
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there is evidence that FGFR-2 IIIb expression sensitivity to FGF7, whereas if the IIIC isoform is expressed, the cancer cells, as it is in the normal cells, they will retain pressed is again not known. If the IIIb isoform is expressed in poorly differentiated cancers. The FGFR-2 isoform being expressed in increasing percentage of FGFR-2-expressing cases in the more expressed detectable FGFR-2, and there was an progression.

Expression of FGFR-2 was not detected in normal luminal epithelial cells by immunohistochemistry. Cultured prostatic epithelial cells express low but detectable FGFR-2 mRNA; thus it is not clear whether there is induction of FGFR-2 expression during culture or whether the concentration of the FGFR-2 in luminal epithelial cells in vivo is below the detection sensitivity of immunohistochemistry. Twenty percent of the well-differentiated cancers expressed detectable FGFR-2, and there was an increasing percentage of FGFR-2-expressing cases in the more poorly differentiated cancers. The FGFR-2 isoform being expressed is again not known. If the IIIb isoform is expressed in the cancer cells, as it is in the normal cells, they will retain sensitivity to FGF7, whereas if the IIIC isoform is expressed, the cells should respond to FGF2 (23). In the Dunning rat prostate cancer system, there is evidence that FGFR-2 IIIb expression inhibits neoplastic progression (28). Carstens et al. (29) have shown that in the DU145 cell line and in three prostate cancer xenografts, there was increased expression of the FGFR-2 IIIc isoform, which is not detectable in normal prostatic epithelial cells in culture, consistent with the idea that exon switching to the FGFR-2 IIIc isoform may also occur in human cancers. It will be important to determine whether there is isoform switching of FGFR-2 in vivo in human prostate cancers, particularly those with increased FGFR-2 expression. Thus, the effect of increasing FGFR-2 expression may be to increase the response to FGF2, if the IIIc isoform is expressed, or to increase response to FGF7 if there is retention of IIIb isoform expression.

Our finding that FGFR-1 and FGFR-2 expression are increased in poorly differentiated prostate cancers is similar to findings reported for a number of different types of carcinoma. Increased expression of FGFR-1 as assessed by immunohistochemistry is strongly correlated with increased tumor stage both in non-small cell lung carcinoma (22) and in head and neck squamous cell carcinoma (17). Expression of FGFR-2 was also increased in advanced head and neck squamous carcinomas (17). It should be noted that both of these studies were performed using the same FGFR antibodies used in this report. A correlation of increased FGFR-1 expression with decreased survival was observed by Ohta et al. (30) in pancreatic carcinoma using a different antibody. Thus in a variety of human cancers, increased FGFR expression is correlated with aggressive disease.

In summary, we have shown that the content of FGF2 in human prostate cancers is increased because of enhanced production by peritumoral stromal cells. Prostatic epithelial cells in culture respond mitogenically to FGF2, and we have found that poorly differentiated cancers express increased levels of FGFR-1, which would enhance their response to FGF2. FGFR-2 is also expressed at increased levels in poorly differentiated prostate cancers, which could again enhance the response of these cancers to either FGF2 or FGF7, which is also present in the cancers at substantial concentrations. However, it is recognized that although the presence of increased growth factor and receptor concentrations in the cancers are consistent with paracrine stimulation of growth and/or invasion, it does not prove that such stimulation is essential for tumor progression. Our current approach to determining whether these interactions are critical in tumor progression is to use knockout mice with either tissue-specific or whole-mouse deletion of these genes to determine whether the progression of prostate cancer in transgenic mouse models is affected when genes such as FGF2 are deleted.

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Fig. 7 Western blot analysis of FGFR-1 and FGFR-2 in prostate cancers. Western blot analysis of tissue extracts from six prostate cancers using either FGFR-1 or FGFR-2 antibodies were performed as described in “Materials and Methods.” The expression of FGFR-1 or FGFR-2 in the cancer cells as determined by immunohistochemistry before Western blot analysis is indicated below each Lane by the + or – sign. The exposure time for the FGFR-1 blot was shorter than that for FGFR-2; therefore, the bands appear to be equivalent, but at equal exposure times, the FGFR-1 band was significantly more intense than the FGFR-2 band.

and metastasis by abrogating the need for stromal factors that are abundantly present in the clinically localized cancers, as we have shown in this report. The same group has shown that FGFR-1 expression enhances tumor progression in this system (27), which supports the idea that the acquisition of FGFR-1 expression that we observed in poorly differentiated human prostate cancers may have an important function in tumor progression.

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