Fibroblast Growth Factor Receptor 2 Limits and Receptor 1 Accelerates Tumorigenicity of Prostate Epithelial Cells

Shuju Feng, Fen Wang, Akio Matsubara, Mikio Kan, and Wallace L. McKeehan

ABSTRACT

Progressive loss of the differentiated phenotype and communication with stroma accompanies the transition of nonmalignant rat prostate epithelial cells to anaplastic, malignant tumors. Here we show that cell surface expression of the fibroblast growth factor receptor 2 (FGFR2) tyrosine kinase is reduced in malignant tumor cell populations (type II) and undetectable at the mRNA level in 30% of cells. This is in addition to the irreversible loss by splice switching of the FGFR2 ectodomain that abrogates response to FGF-7 and homologues from the stroma. One hundred % of type II malignant cells express FGFR1, which is normally expressed in the stroma. Expression of the FGFR1 kinase in premalignant type I tumor epithelial cells by transfection accelerated progression to the malignant phenotype. In contrast to the FGFR2 kinase fused to the ectodomain of FGFR1, the FGFR1 kinase failed initially to support a mitogenic response to FGF-2 in type I tumor cells. However, the FGFR1-transfected cells acquired a mitogenic response after extensive proliferation of the cell population. Resident FGFR2 and ectopic FGFR1 appeared to be partitioned in the type I cells, because neither full-length nor truncated isoforms of FGFR1 affected the mitogenic response of the other. Restoration of the FGFR2IIIb kinase to malignant cells expressing FGFR1 depressed tumor growth rates, restored responsiveness to stromal cells, and restored epithelial cell differentiation. These observations reveal that homologous FGFR1 and FGFR2 kinases play very different roles in cell growth differentiation and in development and support of the malignant phenotype.

INTRODUCTION

A central problem in management of prostate cancer is understanding the transition of relatively benign, slowly growing, differentiated, androgen-sensitive tumors into rapidly growing, anaplastic, hormone-insensitive malignant tumors. Throughout this study, the former are referred to as pre- or nonmalignant type I tumors and the latter as malignant type II tumors rather than nonmetastatic and metastatic tumors, because metastasis was not studied in detail in this report. Differentiated androgen-responsive rat tumors in the Dunning R3327 series, which can be maintained as s.c. implants in syngeneic males, undergo the transition when hosts are subjected to androgen ablation (1). During the process, distinct changes occur in expression of members of the FGF family of polypeptides and FGFR kinases (2–5). This includes a switch from exclusive expression of the IIIb splice variant of the FGFR2 gene in the type I tumor epithelial cells to exclusive expression of the IIIc isoform in type II tumors (5). FGFR2IIIb in epithelial cells is the receptor for the androgen-regulated paracrine signal carried by FGF-7 (keratinocyte growth factor) and its homologues from the stromal compartment (5, 6). FGF-7 binds only FGFR2IIIb, whereas FGFR2IIIc recognizes other FGF polypeptides (5–7). In addition to the switch from exclusive FGFR2IIIb to FGFR2IIIc expression, abnormal (ectopic) expression of the FGFR1 gene, which is normally exclusively found in the stromal compartment, occurs in the type II tumor epithelial cells along with elevated expression of FGF-1, FGF-2, FGF-3, and FGF-5 (2, 5). FGF-1 expression is very low, and FGF-2, FGF-3, and FGF-5 are absent in normal prostate and nonmalignant type I tumor epithelial cells. When implanted in rat hosts in the absence of stromal cells, cloned epithelial cells from the type I tumors progress to type II tumors. The resultant type II tumors exhibit the identical phenotype of type II tumors that evolve from the parent type I tumor after androgen ablation (5). However, when type I tumor epithelial cells are implanted together with type I tumor stromal cells, the progression to type II tumors is prevented, and slowly growing, differentiated, nonmalignant tumors with a well-defined stromal and epithelial compartment are the result (5). The cloned type I tumor epithelial cells, the cellular and molecular properties of which can be dissected and manipulated in vitro, are thought to represent the epithelial cells in the parent Dunning R3327PAP tumor, which evolve into type II tumors after androgen ablation. We have proposed that the loss of the directional paracrine signal carried by FGF-7 from stromal cells to FGFR2IIIb in the epithelial cells of type I tumors may allow subsequent events to unfold that drive progression of the type II tumor phenotype (5). One of those events by correlation appears to be the gain of a new autocrine loop between ectopically expressed FGFR1 and FGF-2, which supports the malignancy that is observed in malignant tumor cells (5). Differences in mitogenic signaling and proteins phosphorylated have been reported between FGFR3 and FGFR4 and FGFR1 and FGFR2, but none have been reported between FGFR1 and FGFR2 (8–11). In this study, we compare the effect of FGFR1 and FGFR2 isoforms on development and reversal of the type II tumor phenotype when transected into nonmalignant type I or malignant type II tumor epithelial cells, respectively. The results show that, despite the high sequence and structural homology, the FGFR2 and FGFR1 kinases play very different roles in epithelial cells in the progression to malignancy and maintenance of the malignant cell phenotype. This suggests that the two FGFRs couple to different signal transduction pathways that affect mitogenesis and phenotype in different cell types.

MATERIALS AND METHODS

Cell Culture and Mitogenic Assays. Primary cultures and stock cultures of cloned cell lines from rat prostate, the Dunning R3327PAP and R3327AT3 tumors, and tumors reconstituted from cell lines were prepared and maintained by described methods (5, 12). Mitogenic activity was assayed by DNA synthesis in cells at low cell density and in serum-free medium. Cells (2 × 10^6) were inoculated into each well of 24-well culture plates in culture medium containing 5% fetal bovine serum for 12 h, and the medium was replaced with new medium containing 0.5% fetal bovine serum for 24 h and then with serum-free medium for 72 h. The cells were washed twice with PBS followed by addition of 500 μl of medium containing 2 μg/ml heparin and 4 ng/ml of the recombinant FGF indicated in the text. After incubation at 37°C for 16 h, 5 μl of [methyl-3H]thymidine (50 μCi/ml, DuPont NEN Research Products, Boston, MA) were added. After 4 h, cells were washed with PBS, fixed in 1

Received 6/5/97; accepted 10/3/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.}

1 To whom requests for reprints should be addressed at Center for Cancer Biology and Nutrition, Albert B. Alkek Institute of Biosciences and Technology and Department of Biochemistry and Biophysics, Texas A & M University, Houston, Texas 77030-3303.

2 Present address: Department of Urology, Hiroshima University School of Medicine, 1-2-3 Kasumi, Hiroshima 734, Japan.

3 This work was supported by NIH Grants CA59971 from the National Cancer Institute.

4 The abbreviations used are: FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; S-E, stromal-epithelial; NPE, normal prostate epithelial; DT, Dunning tumor; DTE, DT epithelial; DTS, DT stromal; Kd, dissociation coefficient.
ml of 5% trichloroacetic acid at room temperature for 30 min, washed with PBS, and extracted with 200 µl of 0.5 N sodium hydroxide. Incorporated [3H]thymidine was counted by liquid scintillation. Representative experiments presented in the text were repeated at least three times and plotted from data points that were the means of triplicates.

**Recombinant FGFR cDNAs and Transfection of Prostate Tumor Cells.** Full-length recombinant constructs are summarized schematically in Fig. 1. Human FGFR1a1, FGFR1β1, FGFR1α2, FGFR1β2, and FGFR2βIIIB1 were prepared as described (4, 13, 14). Full-length rat FGFR2βIIIc1 was generated by ligation of cDNA coding for the extracellular domain of the rat FGFR2βIIIc (GenBank accession no. L19111) and the type I intracellular domain of FGFR2 (GenBank accession no. L19107) at the EcoRI site. The FGFR2βIIIBTr residues 1–418 was generated in the PCR with human sense primer pB1 (13) and antisense primer pB2 (GCGCTAATGATGAGAGCCGAGCCCGTG). Rat prostate cDNA template. cDNAs were cloned into pBluescript SK vector for restriction enzyme and sequence analysis and then cloned into mammalian expression vector pcDNA1/neo (Invitrogen Co., San Diego, CA, 92121) at the HindIII and XbaI sites.

The chimeric cDNA FGFR1β/R2 was constructed by ligation of the extracellular region of human FGFR1β to the intracellular part of rat FGFR2 at a KpnI site with a link generated by PCR with sense primer R21 (CGAATGAA-GGCGGTGTTGGCCAAAGCAGCA) and antisense primer R22 (CAGGGGTTCCTCGCCGGCGGTTCAGCTTG). Rat prostate cDNA template. The PCR product was then cloned into SK vector, sequenced, and excised with KpnI and BamHI for subsequent construction of the chimeric cDNA (Fig. 1). The chimeric FGFR1β/R2 cDNA was then generated by ligation of the extracellular region of rat FGFR2βIIIc to the intracellular part of human FGFR1 at the KpnI site with a link generated by PCR using sense primer R23 (GCTGCTAATGGAACGCGCCTCTACT) and antisense primer R24 (TCGCTTGTGGTACCGTTCTACAT) and rat FGFR2βIIIc1 template. The PCR fragment was cloned into SK vector for sequence verification and excised with EcoRV and KpnI for the construction of the chimeric cDNA (Fig. 1). Chimeric cDNAs described above were cloned into the pcDNA1/neo expression vector between Xhol and XbaI sites.

Transfected cell lines were prepared from cloned lines of type I tumor epithelial (DTE) cells derived from the Dunning R3327PAP tumor and type II tumor (AT3) cells derived from the Dunning R3327AT3 tumor. Cells (5 × 10⁵) in 25-cm² culture flasks were washed twice with PBS and maintained in 1.5 ml of serum-free medium. A mixture of 5 µg of cDNA coding for FGFRI and FGFR2 in the pcDNA1/neo vector and 15 µl of lipofectamine (Life Technologies, Inc., Grand Island, NY) in 100 µl of PBS was added. Cells were incubated at 37°C for 24 h. Transfected cells were selected in medium supplemented with 2% FCS and 400 µg/ml geneticin (Life Technologies, Inc.). Transfected cultures were assessed for expression of sufficient levels of FGFR in radioreceptor assays using FGF-1, FGF-2, or FGF-7 (14, 15).

**Immunohistochemical Analysis of the FGFR1 and FGFR2 Ectodomains.** FGFR-1 (15), FGFR-2 (15), and FGFR-7 (16) were obtained, iodinated, reactivated by reduction, and purified to a specific activity of 2–5 × 10⁶ cpm/ng. Radiolabeled FGF was bound specifically and covalently cross-linked to cell surface FGFR as described (15, 16). Immunohistochemical analysis of complexes covalently labeled with FGF was carried out with rabbit polyclonal anti-FGFR1 serum A50 (4), anti-FGFR2 serum prepared against the ectodomain (17), and anti-FGFR2 serum R2T1 prepared against a synthetic peptide spanning the sequence Pro²⁰¹–Lys²¹ in the COOH-terminal domain of FGFR2 type 1. Immuno-plexes were analyzed by 7.5% SDS PAGE and autoradiography.

**Clonal Analysis of Receptor Phenotype by the PCR.** Clonal cultures from single cells were prepared by dilution of wild-type suspensions of type I tumor (DTE) epithelial cells and type II tumor (AT3) cells into 96-well plates. Expression of the FGFR1 and FGFR2 mRNAs as well as specifically the FGFR2βIIIB and FGFR2βIIIc isomers were analyzed in 50 clonal cultures from each of the DTE and AT3 populations using PCR. Total RNA was extracted from each well, and a first-strand cDNA template was generated by reverse transcription. A 1 µl sample containing 10 ng of cDNA was used as a template in a 100-µl reaction mixture at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 40 cycles. PCR products were run on 2% agarose gels in 0.5× Tris-borate EDTA buffer and visualized with ethidium bromide. Primers yielding the band given (in bp) in parentheses were as follows: FGFR1 (307 bp), FGFR2 (273 bp), FGFR2 (380 bp), R2P3C (5'-TGGCAGAACTGTCAACCATGC-3'); FGFR2βIIIc (380 bp), R2PM (5'-AACGCCGAGGGTGGTGACACG-3'); and R2P3B (5'-GGACGTTACCTGGGTACACGGC-3').

**Tumor Cell Implantation and Histological Analysis.** Prior to use in implantation experiments, clonal cultures were derived from wild-type and transfected cell populations by limiting dilution and selected for expression of the desired type and level of FGFR by screening in radioreceptor assays. At least 50–100–150-g male Copenhagen rats were implanted s.c. in both flanks with 1 × 10⁶ DTE cells, 1 × 10⁵ AT3 cells, or 3 × 10⁵ stromal-derived DTS cells (5), as indicated in the text. Animals were examined regularly for appearance and progress of tumors over a 6-month period for implants using type I tumor epithelial (DTE) cells and 1 month for type II tumor (AT3) cells. For histochemical analysis, fresh tumor tissues were fixed in 10% polyformaldehyde in PBS and embedded in paraffin. Tissues were sectioned and stained with H&E.

**Analysis of Cytokeratins.** A portion (100 mg) of freshly excised tumor tissue was homogenized in 0.5 ml of 1% Triton X-100 and protease inhibitors (17). Cell monolayers were extracted directly with the same solution. After clarification by centrifugation at 16,000 X g, the detergent-soluble fraction was precipitated with H&E.

**RESULTS**

Expression and Activity of FGFR2 and FGFR1 Differ in Premalignant and Malignant Cells. Normal rat ventral prostate cells and the premalignant type I tumor R3327PAP and derived epithelial cells (NPE and DTE, respectively; Refs. 3 and 12) from both tissues express exclusively the IIIb splice variant of the FGFR2 gene (Ref. 5; Fig. 2 and 3). In normal or premalignant epithelial cells, the
FGFR1 AND FGFR2 IN PROSTATE TUMORS

Fig. 2. ^125I-FGF-affinity labeled FGFR complexes in prostate cells. A. ^125I-FGF-1-labeled FGFR2 complexes. Normal rat prostate epithelial cells (NPE), epithelial cells from the Dunning R3327PAP tumor (DTE), cells from late-stage type II tumors derived from cloned DTE cells (E-C, Ref. 5), Dunning R3327AT3 tumor cells (AT3-C), two independently transfected cultures ([1] and [2]) of cells derived from AT3 tumors transfected with FGFR2IIIb1 (AT3-R2IIIb1) and AT3 cells transfected with kinaseless truncated FGFR2IIIb1 (AT3-R2IIIb1Tr) were affinity labeled with ^125I-FGF-1, immunoprecipitated with anti-FGFR2 serum, and analyzed by SDS-PAGE and autoradiography. B. ^125I-FGF-1-labeled FGFR1 complexes. NPE, DTE, E-C, and AT3-C cells described in A; DTE cells transfected with FGFR1a1 (DTE-R1a1); DTE cells transfected with FGFR1b1 (DTE-R1b1); and AT3 cells transfected with FGFR1b1 (AT3-R1b1) were affinity labeled with ^125I-FGF-1 and immunoprecipitated with anti-FGFR1 serum. C. ^125I-FGF-2-labeled FGFR complexes. DTE cells were transfected permanently with control vector (DTE-CV), FGFR1b1 (DTE-R1b1); FGFR1b2 (DTE-R1b2), a chimera comprising the FGFR1b1 ectodomain and the FGFR2 intracellular domain (DTE-R1b2Tr), FGFR2IIIc1 (DTE-R2IIIc1), and the chimera made from the FGFR2IIIc ectodomain and the FGFR1 intracellular domain (DTE-R2IIIc/R1). Untransfected AT3 (AT3-C) cells are also shown. The transfected cells were affinity labeled with ^125I-FGF-2 and analyzed by SDS-PAGE and autoradiography.

FGFR2IIIb recognizes FGF-1 and stromal cell-derived FGF-7, but not FGF-2 (5, 6). A clonal analysis of the expression of FGFR2 and FGFR1 mRNA in premalignant DTE cell populations indicated that all cells express the FGFR2IIIb mRNA (Fig. 3A). Type II tumors and cells that evolve in vivo from parent type I tumors after castration of hosts (1, 5) exhibit a total loss of expression of FGFR2IIIb with a less significant change in expression of total FGFR2 mRNA (5). Type II tumors (E tumors) that emerge from I tumors after castration of hosts (1, 5) exhibit a total loss of expression of FGFR2IIIb with a less significant change in expression of total FGFR2 mRNA (5). A clonal analysis of expression of FGFR2 and FGFR1 mRNA in AT3 cell populations revealed that about 70% of cells expressed the FGFR2 gene (Fig. 3B). The other 30% were completely devoid of mRNA coding for FGFR2 isoforms. Of the 50 clonal cultures analyzed, none exhibited an FGFR2IIIb. In contrast, all 50 cultures derived from single AT3 cells expressed the FGFR1 mRNA (Fig. 3B). These results suggested that a reduction in total cell surface FGFR2 occurs in the malignant cell population. Some cells exhibit a complete loss of expression of the FGFR2 gene in addition to the loss of expression of FGFR2IIIb by splice switching.

Expression of the FGFR1 gene correlates closely with the malignant phenotype of type II tumors that emerge either from parent type I tumors after castration or cloned type I tumor epithelial cells (5). Therefore, we examined the effect of transfection of the FGFR1 tyrosine kinase into premalignant DTE cells on the mitogenic response to FGF-1, FGF-2, and FGF-7 in vitro and their progression to malignancy in vivo. Untransfected DTE cells display about 12,000 FGF-1 receptor sites per cell with an apparent Kd of about 40 pm and no sites for FGF-2 (2). Radioreceptor assay and covalent affinity labeling of receptors (Fig. 2C) were used to identify transfected cells expressing about the same number of new FGF-2 binding sites. FGF-2 bound to the transfected full-length FGFR1a1 (not shown) or FGFR1b1 kinases on the surface of cells (Fig. 2C) but failed to respond to it (Fig. 4). This showed that the FGFR1 intracellular kinase domain is incapable of eliciting a mitogenic response in the DTE cells and strongly supports the thesis that the mitogenic response to FGF-1 and FGF-7 in wild-type DTE cells is mediated by the FGFR2IIIb kinase. The lack of effect of overexpression of FGFR1 constructs with or without a kinase domain on the mitogenic activity elicited by FGF-1 or FGF-7 in the DTE cells (Fig. 4) indicated that neither the ectodomain nor the kinase domain of transfected FGFR1 interacts with the resident FGFR2IIIb. However, it was observed that the mitogenic response to FGF-1 and FGF-7...
FGF1 AND FGR2 IN PROSTATE TUMORS

Fig. 3. Clonal analysis of the expression of FGR2 and FGF1 mRNA in premalignant and malignant prostate tumor cells. A, DTE cells from Dunning R3327PAP tumors. B, AT3 cells from Dunning R3327AT3 tumors. Cells were isolated and purified, and clonal cultures were derived from single cells by limiting dilution. Expression of the indicated FGR isoforms was assessed by PCR from 50 clonal cultures, of which 6 representatives (Lanes 1–6) are displayed. N, negative control in which template cDNA was omitted; P, positive control containing the indicated FGR cDNA; M, markers.

of cells transfected with FGR2βIIIC/R1 (Fig. 4) or FGR2βIIIB/R1 (not shown) and the βIIb or βIIIC isoforms of kinase-defective FGR2β (not shown) was reduced notably.

The Response to FGF-2 in Nonmalignant Epithelial Cells Expressing Recombinant FGR1 Is an Acquired Property. We determined that DTE cells can acquire responsiveness to the FGR1 kinase prior to the loss of FGR2βIIIB and acquisition of the phenotype exhibited by malignant E or AT3 tumor cells. The DTE cells transfected with FGR1β1 [DTE-R1β1(P)] that initially showed no response to FGF-2 were subcultured serially 20 times at 1:5 dilutions of the cell population over a period of about 100 days (about 100 population doublings). The mitogenic response of the DTE-R1β1(P20) cells to FGF-1, FGF-2, and FGF-7 was then compared to that of the parent DTE cells, DTE cells that were transfected with the control vector (DTE-CV), and DTE cells that were transfected with kinase-defective FGR1β2 [DTE-R1β2(P20)]. In contrast to the parent untransfected DTE and DTE cells expressing FGR1β1 just after emergence from selection medium [DTE-R1β1(P1)], the DTE-R1β1(P20) cells acquired a response to FGF-2 without a change in the response to FGF-1 or FGF-7 (Fig. 4). No change in mitogenic response to the three FGF polypeptides was observed in DTE cells expressing kinase-defective FGR1β2 that were cultured in parallel for the same period. These results show that the mitogenic response to the FGR1 kinase in premalignant DTE cells is an acquired property that can occur prior to loss of FGR2βIIIB and consequently prior to the loss of response to stromal-derived FGF-7.

Acceleration of the Malignant Phenotype by Expression of FGR1. We tested whether the presence of the FGR1β1 kinase accelerated the rate of progression to the malignant type II tumors in the absence of stroma (5). After 6 months, DTE cells transfected with the FGR1β1 cDNA gave rise to tumors (DTE-R1β1) that were on average nearly 10 times the wet weight of those that arose from untransfected DTE cells in the same time period (Fig. 5; Table 1). DTE cells transfected with the control vector (DTE-CV) or the kinase-defective FGR1β2 construct yielded tumors that were similar in size to those from untransfected DTE cells.

Restoration of Expression of the FGR2 Gene in Malignant Type II Tumor Cells Reduces Growth Rate of Derived Tumors. Malignant Dunning R3327AT3 (AT3-C) cells were transfected with the FGR2βIIIB1 cDNA (Fig. 2A). Untransfected AT3 cells display about 19,000 FGF-1 and FGF-2 binding sites per cell with an apparent $K_d$ of about 50 pm (2). By radioreceptor assay, transfected cells were selected that acquired one to two times the same number of FGR2 binding sites of similar affinity (Fig. 2A). Separate experiments confirmed that the transfected cells acquired the ability to bind FGF-7 proportional to the number of transfected receptors (not shown). As described previously (1, 5), wild-type AT3 cells (Fig. 6) or their E tumor cell counterparts (not shown) give rise to rapidly growing tumors, which result in multiple organ metastasis and kill the hosts in about 2 months (5). Implantation with stromal cells had no effect on the growth and malignancy of tumors derived from AT3 or E tumor cells (5). In contrast, the size of tumors resulting from AT3 cells expressing FGR2βIIIB1 (AT3-R2βIIIB1 tumors) was on average
Fig. 5. FGFR1 accelerates tumorigenicity of premalignant type I tumor epithelial cells. Untransfected DTE or DTE cells (1 x 10^6) that were transfected with control vector (DTE-CV), FGFRI (DTE-R1β2), and FGFR1 (DTE-R1β3), respectively, were implanted s.c. into the flank of male Copenhagen rats. Tumors were excised and weighed after 6 months. The tumors shown are representative of five animals each (Table 1).

Table 1  Effect of FGFR isoforms on size of tumors

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Tumor weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTE</td>
<td>5.5 ± 2.1</td>
</tr>
<tr>
<td>DTE-CV</td>
<td>4.7 ± 1.8</td>
</tr>
<tr>
<td>DTE-R1β2</td>
<td>6.3 ± 1.5</td>
</tr>
<tr>
<td>DTE-R1β1</td>
<td>51.0 ± 8.9</td>
</tr>
<tr>
<td>AT3</td>
<td>37.8 ± 6.4</td>
</tr>
<tr>
<td>AT3-CV</td>
<td>38.5 ± 5.2</td>
</tr>
<tr>
<td>AT3-R1ββ</td>
<td>14.3 ± 6.1</td>
</tr>
<tr>
<td>AT3-R1β1</td>
<td>35.1 ± 4.8</td>
</tr>
<tr>
<td>AT3-R2βIIIb + DTS</td>
<td>5.7 ± 3.3</td>
</tr>
<tr>
<td>AT3-R2βIIIbTr</td>
<td>12.9 ± 5.4</td>
</tr>
<tr>
<td>AT3-R2βIIIb/R1</td>
<td>37.3 ± 3.8</td>
</tr>
</tbody>
</table>

Data are means ± SE. Five animals were implanted with the indicated cells.
transfected with FGFR1 exhibited predominantly the anaplastic phenotype of the AT3 tumors within 6 months in a single host without additional passage (Fig. 7). AT3 cells transfected with FGFR1β or FGFR2βR1 (not shown) exhibited the histological properties of tumors derived from untransfected AT3 cells (Fig. 7) or those transfected with the control vector (not shown). However, the more slowly growing tumors derived from AT3 cells transfected with FGFR2βIIb1 (AT3-R2βIIb1) exhibited properties much different from those of the parent AT3 tumors. Generally, cells were more densely packed and exhibited more cell-to-cell contacts throughout the tumor than wild-type AT3 tumors (Fig. 7, AT3-R2βIIb1, left). Foci of organized epithelial cells near or actually surrounding blood vessels in the tumor imperfectly resembled the glandlike structures in the DT and DTE tumors (Fig. 7). The anaplastic sheets of cells characteristic of parent AT3 tumors outside the foci of organized cells were infiltrated extensively with lymphocytes, and areas of overt necrosis were apparent. Although tumors resulting from AT3 cells transfected with kinase-defective FGFR2βIIb (AT3-R2IIbTr) were smaller, they exhibited no comparable changes in cellular organization. Rare foci of lymphocyte infiltration and necrosis were observed, but more than 90% of the tumors showed muchilder growth with less extensive lymphocyte infiltration and necrosis.

Expression of the FGFR2 Kinase Restores Expression of Cytokeratins in Malignant Type II Tumors. Both DT tumors (and DTE cells derived from them) and S-E tumors derived from mixtures of DTE and DTS cells expressed a mixture of cytokeratins, whereas total cytokeratins were reduced to undetectable levels in the malignant AT3 and E tumors (reference 5 and Fig. 8). Expression of cytokeratins was reduced in the tumors (Fig. 8, DTE-T) derived from cloned DTE cells and the DTE-R1β1 tumors proportional to their degree of progression toward the type II tumors.
Fig. 7. Histological analysis of prostate tumors. Tissue samples from the parent Dunning R3327PAP tumor (DT) and three indicated tumors resulting from cells transfected with the indicated cDNAs were fixed in 4% polyformaldehyde in PBS overnight, embedded in paraffin, sectioned, and stained with H&E. Magnification, ×200.

pheno...sion. Analyses with individual monoclonal antibodies against keratins 1, 5, 6, 10, 13, and 14 confirmed that AT3 and AT3-R2β1 tumors are negative for all. The DT, DTE, DTE-R1β1, S-E, AT3-R2βIIb, and AT3-R2βIIib+DTS tumors exhibited keratins 5 and 14. Keratin 1 was not detectable in any of the samples, whereas small amounts of keratins 6, 10, and 13 in the S-E tumors and keratin 13 in the DTE-R1β1 tumors could be detected. These results show that restoration of the FGFR2 kinase to AT3 cells restored the expression of cytokeratin in the malignant cells concurrent with the morphological changes described earlier.
Expression of cytokeratin. Extracts of freshly excised tissue from the indicated tumors showed that the ectodomain and the FGFR2 intracellular domain were present to varying degrees. Separate experiments not shown here revealed no difference between growth rates and phenotypic properties of tumors derived from clonal cultures of premalignant type I epithelial cells confirmed that ectopic expression of FGFR1 while FGFR2 is expressed, suggests that it is FGFR1 that supports the malignant phenotype. Transfection of FGFR1 into premalignant type II tumor cells restores lost properties exhibited by their nonmalignant precursors. Accompanying the switch from exclusive expression of FGFR2IIIb to FGFR1IIIc, the reduction in expression of FGFR2 in general, and the activation of FGFR1 in epithelial cells is the loss of both ductal and squamous differentiation and loss of responsiveness to stromal cells. Transfection of the FGFR2IIIb gene into highly malignant type II tumor cells restores some of these properties. The derived tumors not only exhibit a slower growth rate, but they also exhibit changes in cellular organization and cell-to-cell interactions, the absense of which is a hallmark of the anaplastic type II tumors. In addition, the FGFR2IIIb-transfected cells respond to coinoculated type I tumor stromal cells by display of areas of intense keratinization similar to those observed in tumors derived from premalignant type I tumor epithelial cells and stromal cells (S-E tumors; Ref. 5). None of these properties were observed in type II tumor cells transfected with the FGFR1 kinase. These results indicate that the extremely malignant Dunning R3327AT3 tumor cells, which have been selected extensively for malignant and metastatic properties, retain growth-limiting and differentiation-promoting pathways that are responsive to the FGFR2 kinase. The loss of FGFR2 activity, either by lack of activating ligands or by reduction in expression, may be contributory or necessary for progression to and maintenance of the malignant phenotype.

The Relative Importance of FGFR2 and FGFR1 to Prostate Tumor Progression. The results of this study support our previous proposal that FGFR2 plays a self-limiting role in the maintenance of epithelial cell homeostasis directed by the stromal compartment and other environmental factors. In contrast, the stepwise acquisition of the mesenchymal cell property of expression and subsequent activation of FGFR1 may be the positive driving force for progression to and support of the malignant phenotype in the epithelial cell (5). Both changes comprise a loss and acquisition that could cooperate to promote malignancy. It is of interest whether a change in one FGFR causes a change in the other or whether the order of reduction and activation of the FGFR2 and FGFR1, respectively, is important to the rate of malignant progression. Our results suggest that FGFR1 functions independently in premalignant epithelial cells and can accelerate progression to malignancy while coexistent with the resident FGFR2IIIb. However, restoration of FGFR2IIIb1 to the malignant type II tumor cells expressing FGFR1 also appears to override resident FGFR1 in support of the phenotype. Conceivably, the balance between activity of progression-limiting FGFR2 and the progression-promoting FGFR1 determines the rate of progression to malignancy.
Potential Mechanisms Underlying the Different Effects of FGFR1 and FGFR2. Of the four FGFR tyrosine kinases, FGFR1β1 and FGFR2β1 isoforms exhibit the highest similarity. Overall amino acid sequence homology is 72%, 57% in the ectodomains through the beginning of the kinase domains, and 84% in the remainder of the intracellular domain, which includes the kinase and a COOH-terminal domain (20, 21). FGFR2 exhibits tyrosine autophosphorylation sites homologous to those that have been demonstrated in FGFR1 (20, 21). FGFR2 exhibits tyrosine autophosphorylation sites in the intracellular domain, which includes the kinase and a COOH-terminal domain (20, 21). FGFR2 exhibits tyrosine autophosphorylation sites in the intracellular domain, which includes the kinase and a COOH-terminal domain (20, 21). FGFR2 exhibits tyrosine autophosphorylation sites in the intracellular domain, which includes the kinase and a COOH-terminal domain (20, 21). FGFR2 exhibits tyrosine autophosphorylation sites in the intracellular domain, which includes the kinase and a COOH-terminal domain (20, 21). FGFR2 exhibits tyrosine autophosphorylation sites in the intracellular domain, which includes the kinase and a COOH-terminal domain (20, 21). FGFR2 exhibits tyrosine autophosphorylation sites in the intracellular domain, which includes the kinase and a COOH-terminal domain (20, 21).

Thus, it remains to be established how the same FGFR can promote differentiation as a consequence of inhibition of cell cycle promoters was first observed to be associated with differentiation rather than mitogenesis in neuronal cells. From the results, it was proposed that phosphorylation and activation of SNT might indirectly promote differentiation as a consequence of inhibition of cell cycle progression through p130Cas (32). Subsequent analyses indicated that SNT-like proteins are also rapidly phosphorylated in a wide variety of cell types during the mitogenic response to FGF (28). Thus, it remains to be established how the same FGFR can promote mitogenesis, growth limitations, and differentiated phenotypes, as well as how the four homologous kinases cause cell type-dependent differences in the three end points.

The FGFR2βIIIbTr ectodomain without an intact kinase and intracellular domain slows the growth of malignant type II tumors to an extent nearly an equal that of FGFR2βIIIb1. However, the appearance of phenotypic parameters other than reduction in growth rate, e.g., morphological organization, cytotkeratin expression, and response to stromal cells, was dependent on the presence of the FGFR2 kinase. The restoration of the differentiated properties of parent type I tumors in the anaplastic tumor appears to be not simply due to a reduction in tumor cell proliferation rate. The dampening effect of the kinase-defective FGFR2βIIIbTr on tumor growth rate may reflect dimerization between the transgene and endogenous FGFR1 by a dominant-negative mechanism. However, the resident FGFR2 and transfected FGFR1 appear to be partitioned in the premalignant type I tumor epithelial cells and do not interact. Conceivably, the malignant cells have lost the ability to partition the two FGFRs. Recently, we have shown that in the absence of restraints imposed by concentration, heparan sulfate, or other cofactors, the four FGFRs interact promiscuously through a highly conserved interaction interface in the extracellular domain that connects immunoglobulin loops II and III (14, 34). The more dramatic impact of the restoration of the FGFR2βIIIb1 kinase to the growth rate of the type II tumors may reflect dual effects. One effect is the recoupling of the FGFR2 kinase to signal transduction pathways in the malignant cells that limits tumor cell growth and/or promotes differentiation. The other is the suppression of tumor growth rate by heterodimerization with the resident FGFR1 that is driving proliferation and malignancy. It is noteworthy that the FGFR2 ectodomain fused to the FGFR1 intracellular kinase, which presumably homodimerizes with the resident FGFR2 via the ectodomain, dampens FGF-stimulated mitogenesis in nonmalignant epithelial cells. These observations lead to the conclusion that trans-activation events that occur between FGFR intracellular domains within dimers or oligomers (20–23, 35) is homotypic.

ACKNOWLEDGMENTS

We thank Dennis Roop for advice and the analyses with monoclonal antibodies against specific cytotkeratins, Tim Thompson and Hua-shong Zhang for advice and interpretation of tissue sections, and Mei-fang Lu for assistance with preparation of tissue sections.

REFERENCES


Downloaded from cancerres.aacrjournals.org on April 16, 2017. © 1997 American Association for Cancer Research.
Fibroblast Growth Factor Receptor 2 Limits and Receptor 1 Accelerates Tumorigenicity of Prostate Epithelial Cells


Cancer Res 1997;57:5369-5378.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/57/23/5369

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.