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Expression of Basic Fibroblast Growth Factor and Its Receptors FGFR1 and FGFR2 in Human Benign Prostatic Hyperplasia Treated With Finasteride

Carmen Sáez,[1]* Antonio C. González-Baena,[2] Miguel A. Japón,[1] Javier Giráldez,[2] Dolores I. Segura,[1] José M. Rodríguez-Vallejo,[3] Jorge González-Esteban,[3] Gonzalo Miranda,[1] and Francisco Torrubia[2]

[1]*Department of Pathology, Hospital Universitario Virgen del Rocío, Seville, Spain*
[2]*Department of Urology, Hospital Universitario Virgen del Rocío, Seville, Spain*
[3]*Department of Clinical Research, Merck Sharp & Dohme, Madrid, Spain*

BACKGROUND. The development of benign prostatic hyperplasia (BPH) is an androgen-dependent process which may be mediated by a number of locally produced growth factors. One of these, the basic fibroblast growth factor (bFGF or FGF2), has a mitogenic effect on prostatic stroma. High expression levels of bFGF have been reported in BPH. FGFR1 and FGFR2 receptors, that exhibit affinity for bFGF, have been identified in normal and hyperplastic prostate. Finasteride, a 5α-reductase inhibitor, is an effective drug in the treatment of BPH, inducing regressive changes in the prostate of treated patients, even though its mechanisms of action are not yet completely elucidated. This study was designed to assess the effects of finasteride on the expression levels of bFGF, FGFR1, and FGFR2 in patients with BPH.

METHODS. The expression levels of bFGF, FGFR1, and FGFR2 in 9 patients with prostatic hyperplasia treated with finasteride were assessed by immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR) analysis of mRNA expression and were compared with those of 9 control patients with untreated BPH.

RESULTS. Immunohistochemistry showed strong bFGF immunoreactivity in the prostatic stroma of untreated patients, this being somewhat weaker in the epithelium. In treated patients, epithelial immunoreactivity was practically negative, and a considerable reduction in stromal immunoreactivity was seen. These findings were also confirmed by RT-PCR. FGFR1 showed a weak immunoreactivity in the stroma and in basal epithelial cells. FGFR1 showed a weak immunoreactivity in the stroma and in basal epithelial cells. FGFR2 exhibited strong stromal immunoreactivity, becoming weaker in the basal epithelium. No differences were seen in the expression of both receptors between the groups of treated and untreated patients.

CONCLUSIONS. A marked reduction in bFGF levels is seen in BPH treated with finasteride in comparison to untreated BPH. In our opinion, finasteride may act as a negative regulator of bFGF expression, counteracting the role of bFGF in the development of BPH. *Prostate 40:83–88, 1999.*

KEY WORDS: **benign prostatic hyperplasia; bFGF; FGF receptors; finasteride**

*Correspondence to: Dr. Carmen Sáez, Departamento de Anatomía Patológica, Hospital Universitario Virgen del Rocío, Avenida Manuel Siurot, 41013 Seville, Spain. E-mail: dsegura@hvr.sas.cica.es
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INTRODUCTION

Although the mechanisms causing benign prostatic hyperplasia (BPH) are not completely understood, they appear to be androgen-dependent and to be mediated by a number of locally produced growth factors. The role of these growth factors in BPH has been reviewed in several papers [1,2]. One of these factors, basic fibroblast growth factor (bFGF or FGF2), has attracted special attention because it acts as a powerful mitogen stimulating cell differentiation and proliferation, as well as for its angiogenic properties [3]. One of the first growth factors to be isolated in patients with BPH was bFGF [4]. bFGF is synthesized in the fibroblasts of prostatic stroma, where it is found in small quantities in the normal prostate [5,6]. In human BPH, a 2–3-fold increase in bFGF expression, compared to normal prostate, has been noted [7–9]. bFGF binds to receptors with an extracellular region containing three immunoglobulin-like loops and an intracellular domain with tyrosine kinase activity. Four receptors, called FGFR1–4, have been cloned, and their functional diversity has been reviewed by Johnson and Williams [10]. FGFR1 expression occurs in prostatic stromal cells, but not in cultured epithelial cells [6,11]. However, FGFR1 expression has also been documented in basal epithelial cells in vivo [12]. FGFR2 is expressed in considerably smaller amounts in normal prostate, and has been reported in both stromal and epithelial cells in vitro [11]. The alternative or splicing variants, FGFR1 IIIc (*flg* receptor) and FGFR2 IIIc (*bek* receptor), are present in normal prostate and show high affinity for bFGF [6].

The relationship between androgens and bFGF is unclear. Thus, while androgen replacement in castrated rats has been reported to increase bFGF levels [13], in cultured stromal cells androgen supplements do not affect bFGF content [14]. Finasteride (Proscar®, Merck & Co., West Point, PA) is a potent and specific inhibitor of type II 5α-reductase and consequently reduces circulating and intraprostatic dihydrotestosterone (DHT) levels [15,16]. Finasteride is effective in reducing prostate volume and relieving the clinical symptoms of patients with BPH, particularly when the prostate size if 40 g or larger [17–19]. Our study was designed to assess the effect of finasteride therapy on the expression of bFGF and FGFR1 and FGFR2 receptors in human BPH in order to provide further evidence of the relationship between androgens and bFGF in the pathogenesis of BPH.

SUBJECTS AND METHODS

Patients and Tissue Samples

Nine patients with BPH included in treatment protocols with finasteride and 9 control patients with BPH, all with unequivocal criteria for open prostatectomy according to the most widely accepted clinical findings, urine flow abnormalities, and prostate size (more than 50 ml), were selected. The study protocols were approved by the ethical and research committees at our center (Hospital Universitario Virgen del Rocío, HUVR). No patients with permanent bladder catheterization, lithiasis, or any other concomitant vesicoprostatic condition, or patients with any other previous treatment for BPH, were included. Finasteride (5 mg daily) was administered for between 120–168 days. Clinical data of this series were presented previously [20]. Following prostate resection, the specimens were measured and weighed, and representative samples were frozen in liquid nitrogen for subsequent study. Sections adjacent to the frozen samples were fixed in buffered formalin and embedded in paraffin for histologic examination.

Histology and Immunohistochemistry

Histological changes were examined by two pathologists under double-blind conditions. Anti-bFGF, anti-FGFR1, and anti-FGFR2 polyclonal antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were used for the immunohistochemistry study on paraffin-embedded tissue sections. The tissue sections were incubated overnight with the primary antibody (0.5–1 mg/ml) at 4°C. A biotinylated secondary antibody was applied, and the avidine-biotin-peroxidase method was employed to develop the immunostaining, as recommended by the manufacturer (LSAB2 Kit, Dako A/S, Glostrup, Denmark). Diaminobenzidine (DAB) was used as chromogenic substrate for visualization (Biomeda Corp., Foster City, CA). Adequate positive and negative controls were used during the experiments.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

mRNA from frozen prostate samples was isolated using the MicroFast-Track Kit system (Invitrogen BV, Leek, The Netherlands). Using the cDNA Cycle Kit system (Invitrogen), the double-stranded template was synthesized for PCR. Primer sequences for amplification of gene transcripts were obtained from GeneBank and were as follows: bFGF, 5′-TCA AAA GTT CGG CAT GTA G-3′ and 5′-TGG GGA AGA AAT ATC CAT C-3′; FGFR1, 5′-CAT CAA CCA CAC ATA CCA-3′ and 5′-AGT CCG ATA GAG TTA CCC G-3′; and FGFR2, 5′-GTA ACA ACA AGA GAG CAC C-3′ and 5′-ATT CAT TCT CCA CCA CAC-3′. Thirty-five PCR cycles were run in the thermocycler (Mastercycler, Eppendorf-Netheler-Hinz GmbH, Hamburg,

Germany) under the following conditions: 94°C, 1 min; 55°C, 2 min; 72°C, 3 min. The β2-microglobulin gene was coamplified as an internal control. The PCR products were electophoresed in 3% NuSieve agarose gels (FMC BioProducts-Europe, Vallensbaek Strand, Denmark).

RESULTS

Immunolocation of bFGF in Control and Finasteride-Treated BPH

All selected cases corresponded to the mixed glandular/stromal type of BPH. The cases treated showed moderate to marked atrophic changes in the epithelial compartment, in comparison to the untreated control prostates. As a consequence of epithelial atrophy, a relative increase of the stroma was seen in those cases treated with finasteride. A detailed description of the morphological features of this case series was shown previously [20].

The expression of bFGF, FGFR1, and FGFR2 at the protein level was analyzed by immunohistochemistry in representative tissue samples. For adequate comparative analysis, a careful selection of samples was made with similar proportions of epithelium and stroma. The BPH controls showed strong bFGF immunoreactivity in the prostatic stroma, in both the smooth muscle cells and fibroblasts (Fig. 1a). Moderate epithelial immunoreactivity was seen in some hyperplastic glands (Fig. 1b). Stromal immunostaining decreased considerably or was negative in cases treated with finasteride, and no case of epithelial staining was observed (Fig. 2).

Immunolocation of FGFR1 and FGFR2

FGFR1 was mainly located in the stromal fibroblasts, although staining was weak (Fig. 3). FGFR2 was strongly positive in prostatic stroma, with weaker staining found in epithelial cells (Fig. 4). No differences as regards the staining pattern for FGFR1 and FGFR2 were seen between cases treated with finasteride and untreated controls.

Analysis of mRNA Levels of bFGF, FGFR1, and FGFR2 in Cases Treated With Finasteride and Untreated Controls

A close correlation between the immunohistochemical data and the RT-PCR analysis of bFGF mRNA was obtained (Fig. 5a). The bFGF-specific 270-bp band was undetectable in virtually all cases treated with finasteride when coamplified with the β2-microglobulin band. On the other hand, no difference was seen between the patients treated with finasteride and the control cases as regards the mRNA expression of FGFR1 and FGFR2 (Fig. 5b,c).

DISCUSSION

Although the mechanisms leading to the development of human BPH are still largely unknown, there are several findings that suggest that involvement of bFGF in its pathogenesis. Thus, transgenic mice that overexpress the protein int-2, a 27-kDa polypeptide functionally similar to bFGF, develop marked prostatic epithelial hyperplasia [21]. On the other hand, bFGF is a known growth-stimulating factor for prostatic stromal and epithelial cells in vitro [5,22,23]. bFGF is expressed in both normal and hyperplastic prostates, where concentrations are as much as 2 or 3 times higher than in the normal prostate [8,24]. Hamaguchi et al. [12] also found an increase in FGFR1 in prostatic hyperplasia, and suggested that the increase in bFGF and FGFR1 could be due to pathogenetic mechanisms common in the development of BPH. There are a few studies on the location of receptors with affinity for bFGF. Using immunohistochemistry, FGFR1 has been located in basal epithelial cells and in isolated stromal cells, in both the normal and hyperplastic prostate [12]. In RT-PCR studies, the expression of the FGFR1 IIIc and FGFR2 IIIc isoforms, both with a high affinity for bFGF, is reported only in stromal cells [6]. At the time of writing, no papers studying the immunolocation of FGFR2 in normal and BPH prostate have been found in the literature.

Finasteride, an inhibitor of human type II 5α-reductase, is effective in BPH treatment by inducing prostate atrophy with regressive changes in the glandular component [20,25]. In this study, we analyzed the expression levels of bFGF and its receptors FGFR1 and FGFR2 in BPH, and compared these to finasteride-treated BPH, in an attempt to establish a link between the potential role of bFGF in the pathogenesis of BPH and the mechanism of action of finasteride. In our series we showed that bFGF is expressed to a large extent in BPH epithelium and stroma. In specimens from finasteride-treated patients with BPH, no bFGF expression was detected in the epithelial compartment, which shows obvious regressive change after 4–6 months' treatment with the drug. In the stromal compartment, bFGF expression markedly decreased in treated cases, being almost undetectable by the immunohistochemical method used. This reduced bFGF expression in finasteride-treated BPH was confirmed by RT-PCR analysis.

Using immunohistochemistry, FGFR1 was shown to be basically located in the prostatic stroma, with very little immunoreactivity present in the epithelium,

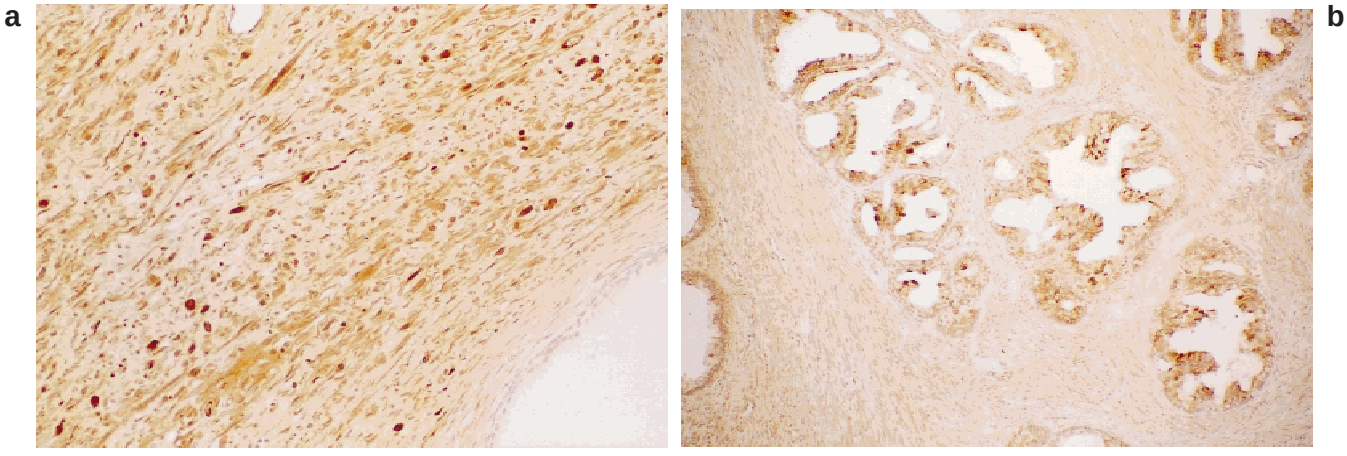

Fig. 1. bFGF immunostaining in control BPH: **a:** Strong immunoreactivity for bFGF in stromal components of the prostate (original magnification, ×50). **b:** Epithelial immunoreactivity was present in some hyperplastic glands (original magnification, ×25).

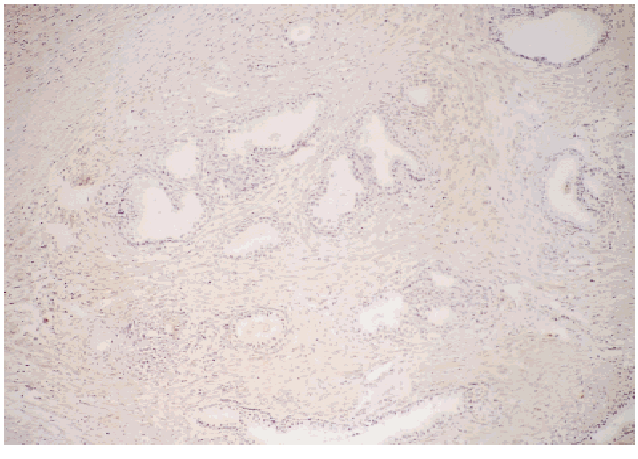

Fig. 2. bFGF immunostaining in finasteride-treated BPH. Cases treated with finasteride showed no epithelial immunoreactivity and a marked decrease of stromal immunoreactivity (original magnification, ×50).

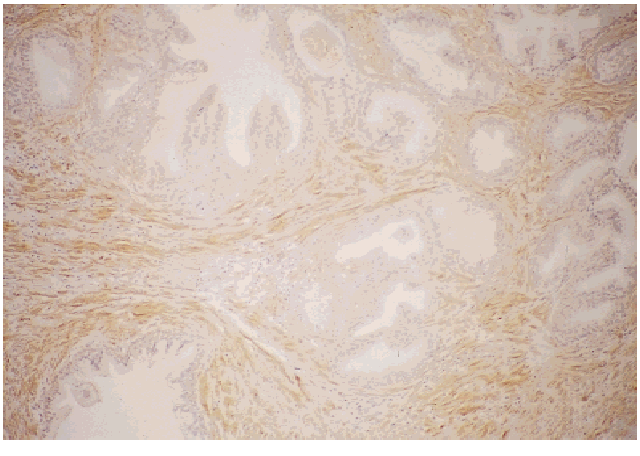

Fig. 3. FGFR1 immunostaining in BPH. Weak immunoreactivity for FGFR1 was detected in stromal cells (original magnification, ×25).

where it was detected in some basal cells. Higher levels of FGFR2 expression were found in the prostatic stromal cells and in basal epithelial cells. An interesting finding was that the immunolocation of both receptors in basal cells occurred preferably in the regressing glands, where the basal cells undergo hyperplastic changes. As regards the RT-PCR evaluation of the expression of these receptors, no differences were found between the cases treated with finasteride and the untreated controls. Since there are no differences between the types of receptors with affinity for bFGF expressed in normal and hyperplastic prostate [11], it would seem that any changes in expression of these FGFRs are not important for the development of BPH.

The reduction in expression levels of bFGF caused by finasteride in BPH specimens confirms to some extent the importance of the role of bFGF in the devel-

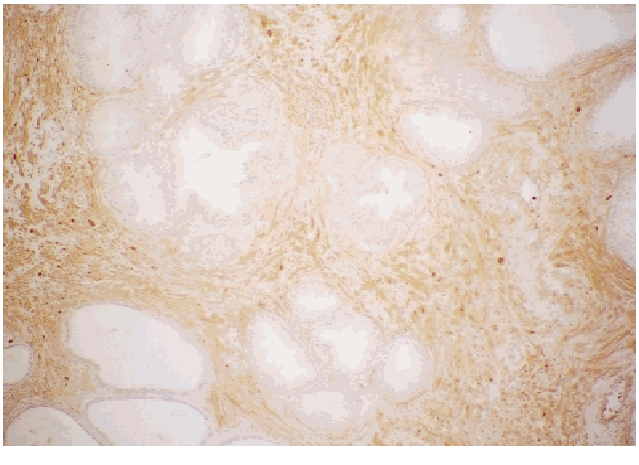

Fig. 4. FGFR2 immunoreactivity in BPH. Signal was strong in prostatic stroma (original magnification, ×25).

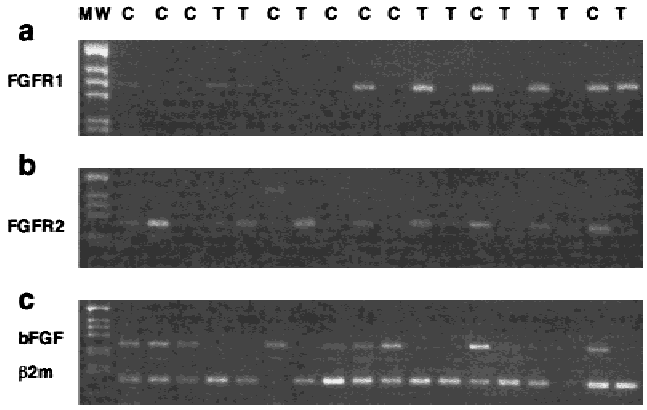

Fig. 5. Analysis of bFGF, FGFR1, and FGFR2 expression by RT-PCR. Amplification of FGFR1 **(a),** FGFR2 **(b),** and bFGF **(c)** RT-PCR products was analyzed on ethidium bromide-stained agarose gels. Cases labeled T correspond to finasteride-treated cases. Controls are labeled C. β2-microglobulin was coamplified in c as an internal control. MW, molecular weight ladder.

opment of this condition. However, one point which remains to be explained is the mechanism by which finasteride causes this decrease in bFGF content. Reduced levels of DHT could negatively affect bFGF transcription. Several studies support a direct effect of androgens on bFGF regulation at the transcriptional level. In the rat, castration reduces the levels of bFGF mRNA in the anterior pituitary, and these are then restored following testosterone administration [26]. In the regressed ventral prostate of castrated rats, transcripts encoding bFGF were reported to increase during the early phase of prostate regrowth in response to testosterone replacement [13]. By contrast, testosterone treatment was also reported to exert a downregulatory effect on bFGF transcription in the rat prostate regrowth model [27] and in T1 rat prostate cancer cells [28].

A final result of the present study was that the levels of FGFR1 and FGFR2 receptors remained practically unchanged. This would lead us to believe that the mechanisms underlying the role of bFGF in BPH pathogenesis are the result of its interaction with other FGF receptors (FGFR3–7) or with receptors as yet undiscovered. This hypothesis was advanced by Story et al. [11] when they failed to find any differences in the expression of these receptors in hyperplastic and normal prostates. They also reported that the mitogenic role of bFGF in the epithelium was much less significant than in the stroma.

In summary, we have presented a series of finasteride-treated cases of BPH in which prostatic atrophy was histologically confirmed. In these treated cases, bFGF expression was either markedly reduced or even undetectable in comparison to the control group of untreated cases. No difference in FGFR1 and FGFR2 expression was seen between the treated cases and controls. We conclude that finasteride could act as a negative regulator of bFGF expression and thus counteract the role of bFGF in the development of BPH. Additional studies are required to assess whether this regulation occurs directly or indirectly through other related regulatory systems.

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