

In Vivo and In Vitro Effects of Androgen on Fibroblast Growth Factor-2 Concentrations in the Human Prostate

Jack Geller, Lida R. Sionit, Andrew Baird, Matthew Kohls,
Kenneth M. Connors, and Robert M. Hoffman

*Mercy Hospital and Medical Center (J.G., L.R.S.), and AntiCancer, Inc., (K.M.C., R.M.H.),
San Diego, and Department of Molecular and Cellular Growth Biology, The Whittier
Institute for Diabetes and Endocrinology, La Jolla (A.B., M.K.), California*

ABSTRACT: Prostatic growth is primarily regulated by dihydrotestosterone (DHT). Recent studies have demonstrated that a large number of growth factors are present in the human benign prostatic hyperplasia (BPH) prostate, including epidermal growth factor (EGF), transforming growth factor- α (TGF- α), transforming growth factor beta (TGF- β), insulin-like growth factor (IGF), and basic fibroblast growth factor (bFGF) (FGF-2). DHT may mediate its mitogenic effects in the prostate by regulating growth factors. To test this hypothesis, we have utilized a histoculture androgen sensitivity assay (HASA) in which ^3H -thymidine incorporation is measured in aliquots of BPH tissue in histoculture with either added DHT or hydroxyflutamide (HF). The resulting DHT/HF ratio is an expression of the androgen sensitivity of the tissue. In this study, we have compared the DHT/HF ratio for ^3H -thymidine incorporation to the DHT/HF ratio for FGF-2 measured in the histocultured prostates. The DHT/HF ratio for the HASA studies of ^3H -thymidine incorporation averaged 2.68 compared to the DHT/HF ratio for FGF-2 in the same specimens of 1.01. These values were significantly different, therefore indicating no relationship between DHT stimulation and FGF-2 levels. In addition, FGF-2 levels were measured in human BPH prostates from patients medically castrated with megestrol acetate and estradiol 17- β prior to surgery. These values were not significantly different, and therefore do not suggest any effect of DHT on the concentration of prostatic FGF-2. Although these studies did not show any effect of DHT on the regulation of prostatic FGF-2, they do indicate that the HASA assay is feasible and appropriate to use in the study of relationships between DHT and various growth factors. © 1994 Wiley-Liss, Inc.

KEY WORDS: flutamide, DHT, ^3H -thymidine, megestrol acetate

INTRODUCTION

The role of growth factors in the regulation of prostatic growth is currently being explored by many investigators. Numerous growth factors and their receptors including insulin-like growth factor 1 (IGF1) [1,2], epidermal growth factor (EGF) [3], transforming growth factor alpha (TGF- α) [3], transforming growth factor beta (TGF- β) [4], and basic fibroblast growth factor (bFGF) (FGF-2) [5] have all been identified in either human benign prostatic hyperplasia (BPH), prostatic cancer tissue, or both. Story et al.

have demonstrated a two-to-threefold increase in the concentration of FGF-2 in BPH tissue and have shown its synthesis by prostatic fibroblasts [6]. This paper goes one step beyond the measurement of the growth factor per se by addressing the question as to whether DHT regulates FGF-2 concentration in BPH tissue.

Received for publication October 29, 1993; accepted February 15, 1994.

Address reprint requests to Dr. Jack Geller, Mercy Hospital and Medical Center, 4077 5th Avenue, San Diego, CA 92103.

We have developed an assay system, the histoculture androgen sensitivity assay (HASA), ideally adapted to the study of interactions between androgens and growth factors [7]. In the previously reported HASA, we demonstrated a predictable and significant increase in ^3H -thymidine incorporation into prostate specimens incubated in histoculture with dihydrotestosterone (DHT) for 5 days as compared to parallel incubations with hydroxyflutamide (HF), which blocks androgen-mediated action [7]. We have used the HASA to study the effect of DHT on FGF-2 by first measuring the ratio of thymidine uptake in DHT relative to HF-treated prostate histocultures. We then compared this ratio to the ratio of FGF-2 in the DHT-treated relative to the HF-treated specimens of the same prostate histocultures. In addition, we have evaluated the possible role of DHT in the regulation of FGF-2 by measuring FGF-2 in fresh untreated BPH tissues, and comparing these values to those obtained from tissues depleted of DHT by medical castration with megestrol plus minidose estrogen (1 mg estradiol 17- β).

MATERIALS AND METHODS

In Vitro Histoculture of Previously Untreated BPH Prostates Obtained at Surgery and Assayed for ^3H -Thymidine Incorporation and FGF-2 Content

Our previously reported 5-day histoculture technique, the HASA, for measuring DHT stimulation of BPH tissue growth was employed in these studies [7]. Briefly, in the HASA, 50–100 mg of minced prostate tissue per well was incubated for 5 days on sponge gel matrices in MEM Earles with additives at a pH of 7.4; aliquots of each prostate studied were incubated with DHT at 2×10^{-8} M or HF at 2×10^{-5} M.

Incorporation of tritiated thymidine, which was added on the fourth day of incubation, served as an indicator of growth stimulation. Incorporation of ^3H -thymidine by DHT-treated specimens was compared to parallel incubations of tissue from the same prostate with HF. We had previously reported that the ratio of thymidine incorporation per microgram of protein was approximately 2.7 times higher in the DHT-treated specimens compared to the HF-treated specimens [7].

Aliquots of the same tissues that were histocultured with DHT or HF and assayed for ^3H -thymidine were also assayed for FGF-2. We also attempted to measure FGF-2 in the conditioned media of the incubates.

FGF-2 Concentrations in BPH Prostates From Patients Treated In Vivo

We measured FGF-2 content by radioimmunoassay in fresh untreated BPH tissue obtained at sur-

gery, and compared the values to those obtained from BPH tissue obtained surgically following 1 week of in vivo treatment with megestrol acetate (MA), 120 mg/day, plus estradiol 17- β (E_2) 1 mg/day. This combination therapy produced a medical castration (plasma testosterone less than 40 ng/dl) as indicated by plasma testosterone levels in all patients. We previously demonstrated that prostate DHT levels in patients who have been medically castrated with this regimen are reduced to less than 1.0 ng/g [8].

Method for extraction and radioimmunoassay

FGF-2 was extracted from histocultured prostates by the method previously described by Gonzalez et al. [9]. Briefly, in this method, tissues were homogenized in a Tris-buffered saline containing enzyme inhibitors at a pH of 7.0. The homogenate was centrifuged at 48,000g for 30 min, then diluted threefold with 10 mmol/L Tris-Cl, pH 7.4, and then incubated at 4°C overnight in the presence of 50 μl heparin-Sepharose. On the following day, the sample was centrifuged and the protein bound to the gel was eluted with two washes of 100 μl of 2 M NaCl in 10 mmol/L Tris-Cl, pH 7.4. The eluted material was then radioimmunoassayed for FGF-2 and corrected for protein concentration.

Immunologic detection of FGF-2

Western blotting of FGF-2 was performed according to previously described methods with slight modifications [9]. The heparin-Sepharose-associated material prepared as described was eluted from the gel with Laemmli's sample buffer. The eluted material was electrophoresed on 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electroblotted onto nitrocellulose, and incubated with protein A-purified antibody to FGF-2 [10].

Antibodies to FGF-2

Polyclonal antibodies were raised against the 1-24 synthetic fragment of bovine FGF (1–146) [10]. These antibodies detect FGF and do not recognize acidic FGF. IgG fractions were prepared by $(\text{NH}_4)_2\text{SO}_4$ precipitation, purified by passage over a protein A-Sepharose column, and used in the studies reported here. In control studies, the IgG used was preincubated with Affigel-10 alone or with Affigel-10 that had been coupled with recombinant FGF-2 (400 $\mu\text{g}/\text{ml}$). The Affigel was removed by centrifugation, and the supernatants were used in control studies to show that depleted antibodies failed to stain the tissue sections. Identical staining was also observed with an IgG prepared from a polyclonal antibody

TABLE I. Comparison of DHT/HF Ratio for ³H-Thymidine Incorporation/μg Protein, and FGF-2 ng/mg Protein in the Same BPH Prostate Following 5-Day Incubation in Histoculture

Tissue no.	DHT/HF for (³ H-thymidine/μg protein)	DHT/HF for FGF-2 (ng/mg protein)
172	1.5	0.96
179	3.2	1.4
180	2.3	0.38
190	3.0	0.93
199	3.4	1.4
	$\bar{X} = 2.68^*$	$\bar{X} = 1.01$

*Significantly different from 1.01; $P = .003$.

raised in rabbits against intact human recombinant FGF-2.

RESULTS

As shown in Table I, the ratio of tritiated thymidine incorporation/μg protein in the DHT-treated compared to the HF-treated histocultures averaged 2.68. The ratio of FGF-2 in the DHT samples as compared to HF samples of the same five histocultured specimens averaged 1.01. There was no statistical correlation between the DHT/HF ratio for ³H-thymidine incorporation and the FGF-2 ratio for the same specimens. Immunoreactive FGF-2 was undetectable (<200 pg/mg) in the conditioned media from the histoculture experiments (not shown).

In the in vivo studies, FGF-2 concentration was 22.97 μg/mg protein in extracts of tissues from patients pretreated with MA and E₂ (n = 8). This compares to a concentration of 24.65 in the untreated specimens (n = 10) and indicates that there is no significant difference between the two (see Table II). Patients pretreated with MA plus E₂ had plasma testosterone levels on the day of surgery averaging 44 ng/dl which establishes the efficacy of the medical castration.

DISCUSSION

Although there is no doubt that FGF-2 is found in the prostate and is produced by the stromal cells, Story et al. [4] have suggested that its levels may not be regulated by androgen but by TGF-β. Zuck et al. [11] published data indicating that androgens may, in fact, stimulate the release of substances in the FGF family, although they did not indicate that it was FGF-2. In Zuck's experiments, there was evidence that coculture of LnCap cells with SW-13 cells resulted in a stimulation of the latter by the former. This stimulation was markedly augmented by incu-

TABLE II. bFGF Levels in Prostates of Patients Treated With MA and E₂ Compared to Untreated Control BPH Tissues

Controls (bFGF-ng/mg protein)	MA + E ₂ (bFGF-ng/mg protein)
18.08	15.49
9.42	26.91
32.46	10.59
30.32	52.89
11.74	14.54
82.01	32.08
8.84	4.97
16.74	26.29
20.46	
16.28	
	$\bar{X} = 22.97 + 5.36$ S.E.M.
$\bar{X} = 24.65 \pm 6.85$ S.E.M.	

bation with androgen. The fact that anti-FGF antibodies inhibited the growth of the SW-13 cells did suggest that the SW-13 cell growth was mediated by androgen stimulation of FGF-2.

However, Cunha et al. [12] cited data by Alarid et al. [13] that strongly support the thesis that FGF-2 is not a significant factor in mediating prostate growth. Alarid et al. [13] showed that cotransplanted rat urogenital sinuses, representing a prostatic anlage, were unaffected in their growth by specific anti-FGF-2 antisera as compared to specimens treated with normal rat serum in vivo. Cunha et al. [12], in the same article, cited evidence that keratinocyte growth factor (KGF), which is a recently discovered member of the FGF family, may be implicated in prostate growth. In view of the fact that there are now nine members of the FGF family including one characterized as androgen dependent [14], perhaps the effects attributed to FGF-2 are due to the presence of an FGF-2-related protein. The FGFs appear to share receptor cross-reactivity and, as such, it will be important to clearly delineate the potential role of each in prostate growth, development, and disease.

With the data obtained through our histoculture experiments, we have shown that there is no relationship between DHT-stimulated growth in our histocultured experiments and changes in FGF-2. In addition, our in vivo studies, in which we depleted the prostate of androgen with MA and E₂ for a week prior to surgical resection, suggest no effect on the concentration of FGF-2 by decreasing DHT concentration in the prostate.

CONCLUSIONS

Despite the fact that these studies showed no correlation between DHT and FGF-2 levels, it is the first

demonstration of the usefulness of HASA in exploring physiological relationships between hormones and growth factors. A further analysis of the role of other FGFs in this model is underway, even though these studies do not support the thesis that DHT mitogenic effects in human prostate tissue are associated with changes in FGF-2 tissue levels.

REFERENCES

1. Cohen, P, Peehl DM, Lamson G, Rosenfeld RG: Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins in primary cultures of prostate epithelial cells. *J Clin Endocrinol Metab* 73:401-407, 1991.
2. Serio M, Fiorelli G: Dual control by androgens and peptide growth factors of prostatic growth in human benign prostatic hyperplasia. *Mol Cell Endocrinol* 78: C77-C81, 1991.
3. Yang Y, Chisholm GD, Habib FK: Epidermal growth factor and transforming growth factor α concentrations in BPH and cancer of the prostate: Their relationships with tissue androgen levels. *Br J Cancer* 67:152-155, 1993.
4. Story MT, Hopp KA, Meier DA, Begun FP, Lawson RK: Influence of transforming growth factor β 1 and other growth factors on basic fibroblast growth factor level and proliferation of cultured human prostate-derived fibroblasts. *Prostate* 22:183-197, 1993.
5. Story MT: Fibroblast growth factor: Implications in the etiology of benign prostatic hyperplasia. In Karr JP, Coffey DS, Smith RG, Tindall DJ (eds): "Molecular and Cellular Biology of Prostate Cancer." New York: Plenum Press, 1991, pp 81-90.
6. Story MT, Livingston B, Baeten L, Swartz SJ, Jacobs SC, Begun FP, Lawson RK: Cultured human prostate-derived fibroblasts produce a factor that stimulates their growth with properties indistinguishable from basic fibroblast growth factor. *Prostate* 15:355-365, 1989.
7. Geller J, Sionit LR, Connors K, Hoffman RM: Measurement of androgen sensitivity in the human prostate in in-vitro three-dimensional histoculture. *Prostate* 21: 269-278, 1992.
8. Geller J: Megestrol acetate plus low-dose estrogen in the management of advanced prostatic carcinoma. *Urol Clin North Am* 18:83-91, 1991.
9. Gonzalez AM, Buscaglia M, Fox R, Isacchi A, Sarmientos P, Farris J, Ong M, Martineau D, Lappi DA, Baird A: Basic fibroblast growth factor in Dupuytren's contracture. *Am J Pathol* 141:661-671, 1992.
10. Gonzalez AM, Buscaglia M, Ong M, Baird A: Distribution of basic fibroblast growth factor in the 18-day rat fetus: Localization in the basement membranes of diverse tissues. *J Cell Biol* 110:753-765, 1990.
11. Zuck B, Goepfert C, Nedlin-Chittka A, Sohr K, Voight KD, Knabbe C: Regulation of fibroblast growth factor-like protein(s) in the androgen-responsive human prostate carcinoma cell line LNCaP. *J Steroid Biochem Mol Biol* 41(3-8):659-663, 1992.
12. Cunha GR, Alarid ET, Turner T, Donjacour AA, Boutin EL, Foster BA: Normal and abnormal development of the male urogenital tract. Role of androgens, mesenchymal-epithelial interactions, and growth factors. *J Androl* 13:465-475, 1992.
13. Alarid ET, Cunha GR, Young P, Nicoll CS: Evidence for an organ and sex-specific role of bFGF in the development of the fetal mammalian reproductive tract. *Endocrinology* 129:2148-2154, 1991.
14. Tanaka A, Miyamoto K, Minamino N, Takeda M, Sato B, Matsuo H, Matsumoto K: Cloning and characterization of an androgen-induced growth factor essential for the androgen-dependent growth of mouse mammary carcinoma cells. *Proc Natl Acad Sci USA* 89:8928-8932, 1992.