

·Original Article ·

Effect of β radiation on TGF- β_1 and bFGF expression in hyperplastic prostatic tissues

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Abstract

Aim: To investigate the transforming growth factor β_1 (TGF- β_1) and basic fibroblast growth factor (bFGF) expressions in benign prostatic hyperplasia (BPH) and the effect of β -radiation. **Methods:** TGF- β_1 and bFGF expression was studied by means of an immunohistochemical method in nine normal prostatic (NP) tissues, 15 hyperplastic prostatic tissues and 35 hyperplastic prostatic tissues treated with 90 Sr/ 90 Y. **Results:** The TGF- β_1 expression in the epithelium and stroma of normal prostatic tissues was 68.2 % ± 10.5 % and 29.7 % ± 4.6 %, respectively, while it was 64.8 % ± 9.3 % and 28.6 % ± 4.1 %, respectively, in hyperplastic prostatic tissues. Compared with the controls, TGF- β_1 expression in the epithelia and stroma of BPH treated with 90 Sr/ 90 Y increased significantly (*P* < 0.01). The bFGF expression in epithelia and stroma of normal prostatic tissues was 17.4 % ± 3.7 % and 42.5 % ± 6.8 %, respectively, and was 46.3 % ± 8.2 % and 73.2 % ± 12.1 %, respectively, in hyperplastic prostatic tissues. Compared with the controls, expressions of bFGF in the epithelia and stroma of BPH treated with 90 Sr/ 90 Y prostatic tissues. Compared with the controls, expressions of bFGF in the epithelia and stroma of BPH treated with 90 Sr/ 90 Y prostatic tissues. Compared with the controls, expressions of bFGF in the epithelia and stroma of BPH treated with 90 Sr/ 90 Y prostatic tissues. Compared with the controls, expressions of bFGF in the epithelia and stroma of BPH treated with 90 Sr/ 90 Y prostatic hyperplasia applicator decreased significantly (*P* < 0.01). **Conclusion:** Exposure of β -rays had noticeable effects on BPH tissues, enhancing TGF- β_1 expression and inhibiting bFGF expression. (*Asian J Androl 2005 Mar; 7: 49–54*)

Keywords: benign prostatic hyperplasia; radiation; transforming growth factor β_1 ; basic fibroblast growth factor

1 Introduction

Benign prostatic hyperplasia (BPH) is one of the common diseases occurring in older male patients [1]. The generation and development of BPH are associated with the proliferation and apoptosis of prostatic cells. Polypeptide growth factors also play important roles in this procedure. To date, the transforming growth factor β_1

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(TGF- β_1) is the only known growth factor that can suppress tissue proliferation and induce cell apoptosis, while the basic fibroblast growth factor (bFGF) has the opposite effect [2–5]. β -ray causes the prostate to shrink by inducing hyperplasic prostatic cells to putrecfy and undergo apoptosis [6]. This paper is to study the TGF- β_1 and bFGF expression in normal prostate and BPH and the inhibitory effects of β -ray on the expression in hyperplasic prostatic tissues.

2 Materials and methods

2.1 Clinical information

Total fifty BPH cases were chosen and 35 of these

cases were treated with transurethral prostatectomy or transvesical prostatectomy after β radiation. The age of the patients ranged from 57 to 86 (mean 72.3) years. The disease courses were 2 to 23 years with a mean of 7.5 years. The nine normal prostates (NP) were from persons who had died unexpectedly (aged 20 to 31 years, mean 26).

2.2 Urethra-type applicator

The ⁹⁰Sr/⁹⁰Y prostatic hyperplasia urethra-type applicator was designed according to the local anatomy. In addition, its effectiveness, safety and reliability were considered. The hypertrophic prostate gland can be irradiated by 90 Sr/ 90 Y β -rays through the wall of the urethra. The structure of the SRPA-104 (Isotope Division of China Institute of Atomic Energy, Beijing, China) type applicator is shown in Figure 1. It is composed of a stainless steel urinary catheter, a 90Sr/90Y annular source, a shield tube and the controller. In order to fix the irradiation position accurately, a urination porthole is adopted. As the applicator is inserted gradually into the urocyst from the urethral canal, urine will enter the catheter from the porthole and flow out at the end of the catheter. Then, the therapeutic head is moved forward about 12 mm. So, the active region (90Sr/90Y annular source) is just located in the position of the prostate gland section. The dimensions of the SRPA-104 type applicator are 5 mm or 6 mm in diameter and 280 mm long. The surface dose is about 2–4 cGy·s⁻¹. For clinical application, the total surface dose is controlled within 30-40 Gy (for a singletime irradiation).

2.3 Exposure grouping

Fifty BPH patients were divided into two groups: the exposure group and the hyperplasia group. Of the total 35 patients accepting a 30–50Gy surface dose of β ra-



1. Catheter 2.β-source 3.Window 4.Shield 5.Start up 6.Handle

Figure 1. SRPA-104 β -Intracavitary Applicator (urethra-type). Cited from Cai SY, Li Z, Chen F. 90Sr/90Y prostatic hyperplasia applicators and its clinical effects. Proceedings of 10th Pacific Basin Nuclear Conference, Kobe, Japan, 1996, p.1465. diation for about 30 min in the former group, 12 were practiced operation on the 4th day after exposure, another 12 on the 7th day and the rest 11 on the 15th day. 15 cases in the latter group consisted of patients without intracavitary exposure of 90 Sr/ 90 Y β -rays.

2.4 Immunohistochemical staining

TGF- β_1 and bFGF assay kits were purchased from the Neomarkers Co. (Fremont, Calif., USA). All specimens of prostatic tissues were fixed by formalin for 24-48 hours and embedded in paraffin. Then 4 µm sections were cut. All sections were subjected to a paraffin-removal procedure and hydrated sufficiently before the addition of 3 % H₂O₂ and before they were laid up for 10 min, in order to inhibit the activity of endogenous peroxydase. The staining procedure was as follows. The sections were washed with phosphate buffered solution (PBS) (5 min, 3 times). Then, they were put into citrate buffer solution and heated to boiling point for 2-3 times with a time intervals of 5–10 min. After cooling down, (1) the sections were washed with PBS 1-2 times; (2) reagent II was added at an ambient temperature for 10 min and redundant liquids were removed; (3) primary antibodies were added and the sections were placed at an ambient temperature for 1 hour before washing with PBS (5 min, 3 times); (4) reagent III (the second antibody) was added and sections were placed at an ambient temperature for 10 min before washing with PBS (5 min, 3 times); (5) reagent IV (the third antibody) were added and the sections were placed at an ambient temperature for 10 min before washing with PBS (5 min, 3 times); (6) sections were stained with diamino-benzidine and the nuclears were stained with hematoxylin-eosin (HE); and (7) all specimens were hermetizated with neutral glue.

2.5 Assessment of results

The positive substances were located at the cytoplasm as brown and dark brown granules. Staining concentrations in different parts of the tissue section were observed under $\times 100$ magnification. Under $\times 400$ magnification, five randomly selected eyeshots were observed for the positive cell number.

2.6 Statistical analysis

Data were expressed as mean \pm SD and analyzed by Students' *t*-test. *P* < 0.05 was considered as significant.

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Groups	bFGF in epithelial cells (%)	bFGF in stromal cells (%)	
Normal	$17.4 \pm 3.7^{\circ}$	$42.5\pm6.8^{\circ}$	
Hyperplasia	46.3 ± 8.2	73.2 ± 12.1	
4 days after exposure	36.8 ± 5.1^{b}	62.4 ± 10.8^{b}	
7 days after exposure	$20.5 \pm 4.2^{\circ}$	48.9 ± 6.3^{b}	
15 days after exposure	$16.4 \pm 3.2^{\circ}$	$40.1 \pm 6.5^{\circ}$	

Table 1. The effect of 90 Sr/ 90 Y β -rays on bFGF expression in prostatic tissues (mean ± SD, *n*=3). ${}^{b}P < 0.05$, ${}^{c}P < 0.01$, compared with the hyperplasia group.

3 Results

3.1 Positive expression of TGF- β_1

The positive rates of TGF- β_1 expression in NP epithelial cells and stromal cells were 68.2 % ± 10.5 % and 29.7 % ± 4.6 %, respectively while the positive rates of TGF- β_1 expression in BPH epithelial cells and stromal cells were 64.8 % ± 9.3 % and 28.6 % ± 4.1 %, respectively. However, 4 days after intracavitary exposure of ⁹⁰Sr/⁹⁰Y β-rays, the positive rates of TGF- β_1 expression in BPH epithelial cells and stromal cells were 81.3 % ± 14.6 % and 37.5 % ± 6.2 %, respectively, 89.1 % ± 16.5 % and 39.4 % ± 6.7 %, respectively 7 days later and increased to 93.7 % ± 17.2 % and 41.8 % ± 7.3 % 15 days later (Figure 2).

3.2 Positive expression of bFGF

The positive rates of bFGF expression in the NP epithelial cell and stromal cell were 17.4 % ± 3.7 % and 42.5 % ± 6.8 %, respectively and those of bFGF expression in the BPH epithelial cell and stromal cell were 46.3 % ± 8.2 % and 73.2 % ± 12.1 %, respectively. With ⁹⁰Sr/⁹⁰Y β exposure, the positive rates of TGF- β_1 expression in the BPH epithelial cell and stromal cell were reduced gradually and reached the lowest level on day 15 of radiation of exposure (Table 1, Figure 3).

3.3 Results comparison

TGF- β_1 positive expression rates of NP and BPH epithelial cells were higher than those in correspondent stromal cells (P < 0.01). No statistically significant association was found between TGF- β_1 positive expression of epithelium and stroma in BPH tissues and the corresponding expression in NP (P > 0.05). Compared with the hyperplasia group, TGF- β_1 positive cell rates in BPH epithelial and stromal cells were significantly increased 4, 7 and 15 days after 90 Sr/ 90 Y β exposure (P < 0.01). Expression of bFGF existed not only in epithelial and stromal cells of BPH, but also in normal prostatic tissues. The bFGF positive expression rates in BPH epithelial and stromal cells were higher than those in NP epithelial and stromal cells (P < 0.01). bFGF positive expression rates in BPH and NP stromal cells were higher than those in corresponding BPH and NP epithelial cells (P < 0.01). bFGF levels in BPH epithelial and stromal cells decreased sharply 4, 7 and 15 days after 90 Sr/ 90 Y β exposure (P < 0.01).

4 Discussion

Some polypeptide growth factors, such as keratinocyte growth factor (KGF), epidermal growth factor (EGF), fibroblast growth factor (FGF) and insulin-like growth factor (IGF) promote tissue proliferation and play an important role in the generation of BPH. However, TGF- β_1 , a multi-functional growth factor generated by the prostate stromal cells, is the only known growth factor that suppresses tissue proliferation [7]. TGF- β_1 promotes apoptosis of prostatic cells and synthesis of extracellular matrix (ECM). Thus, it suppresses cell proliferation [8]. ECM consists of collagen protein, nectin and proteoglycan. These elements are filled into the gaps between cells and involved in linkage, growth, differentiation and senescence processes. EGF, FGF and IGF suppress the apoptosis-enhancing effects of $TGF-\beta_1$. Some research has indicated that apoptosis could be induced directly when TGF- β_1 is applied to *in vitro* cultured prostatic epithelial cells [9].

bFGF, a representative of the FGF family, adjusts extracellular stromal proteinase activity and accelerates synthesis of collagen and protein polysaccharide. Also, bFGF adjusts remoulding of extracellular stroma and induces cell generation and differentation. bFGF is considered an important cellular karyokinesis acceleration factor and is a revulsant of the collagen enzyme IV; it is involved in the processes of organism growth, vascular





Figure 2. TGF- β_1 expression in the epithelial and stromal cells of prostates, ×400. (A): NP epithelial and stromal cells. (B): BPH epithelial and stromal cells, (C): 4 days after ⁹⁰Sr/⁹⁰Y β exposure in BPH, (D): 7 days after ⁹⁰Sr/⁹⁰Y β exposure in BPH, (E): 15 days after ⁹⁰Sr/⁹⁰Y β exposure in BPH.

formation, tissue rehabilitation, wound healing and certain pathology processes [10].

In recent years, most researches on bFGF in prostatic tissues were focused on the protein and cellular level instead of gene level [6–11]. It has been shown that bFGFmRNA levels in BPH tissues were significantly higher than those in normal prostatic tissues [12]. In BPH tissues, bFGF levels were 2–3 times higher than those in a normal prostate, with significantly higher bFGF mRNA expression [13]. In BPH epithelial and stromal cells, bFGF expression was significantly higher than that in NP. In NP and BPH stromal tissues, bFGF protein positive expression was higher than that in the epithelium. bFGF, a powerful karyokinesis accelerator in prostatic stromal cells, was mainly distributed in the BPH stromal region, and reached its highest level in hyperplastic pros-





Figure 3. bFGF expression in epithelial and stromal cells of prostates, ×400. (A): NP epithelial cells and stromal cells, (B): BPH epithelial and stromal cells, (C): 4 days after 90 Sr/ 90 Y β exposure in BPH, (D): 7 days after 90 Sr/ 90 Y β exposure in BPH, (E): 15 days after 90 Sr/ 90 Y β exposure in BPH.

tate urethra [14]. After an internal exposure to β -rays, the positive bFGF expression in prostate epithelial and stromal cells was reduced. Moreover, significant pathological changes of prostatic tissues were observed after radiation. TGF- β_1 positive expression in prostatic epithelial and stromal cells increased obviously; simultaneously bFGF positive expression reduced remarkably. This resulted in an increase in the TGF- β_1 /bFGF ratio and the

apoptosis of prostatic epithelial cells and stromal cells. This explains how the TGF- β_1 negative regulating effects on prostatic epithelial cells and stromal cells were enhanced, and bFGF positive regulating effects were weakened, which accelerated the apoptosis of prostatic cells and shrinking of tissues [1]. However, β -rays radiation increased the TGF- β_1 /bFGF ratio and induced the corresponding differentiation processes of epithelial

cells and stromal cells.

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