A total of 60 prostate cancer and 20 benign prostate hypertrophy patients were collected for tissue samples during the surgery. The expressions of PTEN and TGF-β in both tissues were detected examined by immunohistochemical (IHC) staining. The mRNA level of PTEN was measured by in situ hybridization (ISH). PTEN gene expression was significantly higher in benign prostate tissues compared to tumors. With advanced grade of tumors, the expression level of PTEN was further down-regulated. IHC results showed significantly elevated expression of TGF-β in prostate cancer tissues compared to hypertrophy tissues. PTEN gene expression was down-regulated in prostate cancer tissues compared to hypertrophy tissues, while TGF-β expression level was potentiated. There was a negative correlation between PTEN and TGF-β levels, both of which may reflect the malignancy of prostate cancer to some extents.

Keywords: Prostate cancer, PTEN, TGF-β, immunohistochemistry, in situ hybridization

Introduction

As one malignant tumor occurred in epithelial cells of male prostate tissue, prostate cancer can be divided into adenoma, ductal adenocarcinoma, urothelium carcinoma, squamous carcinoma and adenosquamous carcinoma based on pathological features [1, 2]. Prostate cancer has a relatively higher incidence in Western countries. In China, however, the occurrence rate is now 9.92 per 100,000 people and is continuously rising [3]. Prostate cancer has a complicated pathogenesis mechanism involving multiple factors including genetic, age, diet habit and sex activity. With recent progresses regarding the molecular biological mechanism of prostate cancer, the occurrence of tumor has been found to be closely correlated with various oncogenes and tumor-suppressor genes [4].

Anti-tumor gene phosphatase and tensin homology (PTEN) locates on human chromosome 10q23 region, and encodes one specific phosphatase with dual specificity including lipid phosphatase and protein phosphatase [5]. Studies have revealed the importance of PTEN in the pathogenesis and progression of prostate cancer, and its correlation with unfavorable prognosis. The study about PTEN gene expression alternation and related functional mechanisms has become the focus of prostate cancer [6]. Transforming growth factor-β (TGF-β) is one type of polypeptide cytokine [7] and can exert different biological effects on various cells. In normal epithelial tissues, TGF-β can inhibit cellular proliferation; while in malignant tumors it can facilitate the proliferation, infiltration and metastasis [8]. This study measured the expression of PTEN and TGF-β in prostate cancer tissues and benign prostate tissues, in an attempt to analyze the correlation between expression...
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Materials and methods

Clinical samples and patients

A total of 60 prostate cancer patients (aging between 58 and 84 years old, average = 71.8 ± 8.3 years) who received surgical resection in Sichuan Academy of Medical Sciences and Sichuan Provincial People’s Hospital from January 2014 to January 2015 were recruited in this study. All patients had not received any chemo-, radio- or immune therapy before the surgery. Prostate adenoma has been confirmed on all patients by pathological examinations. According to WHO classification, there were 11 cases at class I, 12 patients at class II and 37 cases of class III patients. Using Whitmore-Jewett grading scale, there were 14 patients at stage A or stage B, 15 cases of stage C, plus 31 stage D patients. Another cohort of 20 benign prostatic hyperplasia (BPH) patients (aging between 55 and 86 years old, average = 69.6 ± 8.8 years) who received surgical treatment at the same period were recruited as the control group. Tissue samples were collected from both groups during the surgery, and were fixed in 10% neutral buffered formalin (NBF). Tissues were embedded in paraffin block, which was sectioned into 4-μm thickness slices for hematoxylin-eosin (HE) staining, pathological grading, immunohistochemistry (IHC) staining, and in situ hybridization (ISH). This study has been pre-approved by the ethical committee of Sichuan Academy of Medical Sciences and Sichuan Provincial People’s Hospital, and has obtained written consents from all participants.

Reagents

Ready-to-use mouse anti-human PTEN or TGF-β monoclonal antibody was purchased from Shengxing Biotech (China). DAB development kit was a product of Boxin Reagent (China). SP staining kit for IHC was obtained from RD (US). ISH probes were synthesized by Sangon (China).

IHC staining

The expression of PTEN or TGF-β was detected by IHC staining using previously established method [9]. In brief, paraffin tissues slices were de-waxed in xylene, and were re-hydrated in gradient ethanol and distilled water. Endogenous peroxidase activity was quenched by 3% H2O2 for 20 min. After PBS rinsing twice, 10% normal goat serum was applied for 10-min blocking at room temperature. Primary antibody was then added for 2-hour incubation at 37°C. Secondary antibody with biotin labelled was then added for 30-min incubation. After further PBS rinsing, horseradish peroxidase working solution was added for further 30-min incubation. DAB substrates were added to develop tissue slices. Hematoxylin was then added for counter-staining. After dehydration, tissues slices were observed under a microscope. Five high-magnification (400×) fields were randomly selected to count the total number of cells and positive stained cells. Positive staining was identified as brown-yellow granules in the cytoplasm. The overall positive staining was determined as more than 40% of positive stained cells; otherwise cells were deduced as having negative staining.

ISH method

The mRNA level of PTEN was detected by ISH as previously reported [10]. In brief, paraffin-based tissues slices were dewaxed and rehydrated using routine procedures. After 3% H2O2 quenching for 10 min, proteinase K was added for incubation. The tissue slices were rinsed in 0.5 M PBS (pH 7.4) for three times, and were immersed in pre-hybridization buffer under 65°C for 4 hours. The hybridization buffer containing specific RNA probes was then used for hybridization at 65°C overnight. After hybridization, tissues were incubated sequentially by 2XSSC and 0.2XSSC (10 min, for three times each). Rabbit anti-DIG working solution was then added for 60-min incubation at 37°C, followed by PBS washing. Alkaline phosphatase buffer was added for 5-min incubation, with the addition of chromogenic substrates for developing in dark. 4% paraformaldehyde was used to fix tissue slices, which were then observed under a microscope after mounting coverslips. Positive staining was deduced as blue-violet granules in cytoplasm. Overall positive staining was identified when more than 40% of positive stained cells existed. Parallel negative control slices were employed using 0.5 M PBS instead of hybridization probes [11].

Statistical methods

SPSS 17.0 software was used to analyze all collected data, of which measurement data were presented as mean ± standard deviation (SD).
while enumeration data were presented as ratios. Between-group comparison was performed by rank-sum test. Spearman analysis was used for correlation analysis. A statistical significance was define when P<0.05.

Results

PTEN IHC staining

The staining intensity of PTEN was significantly higher in BPH tissues compared to prostate cancer tissues (Figure 1). When comparing staining patterns across different grades, we found higher PTEN-positive rate in grade I and II tissues of prostate cancer than grade III.

TGF-β staining

IHC results revealed opposite patterns of TGF-β against PTEN, as prostate cancer tissues had higher positive rate and staining intensity compared to BPH tissues. In tumor tissues with advanced grade, the staining intensity was even higher (Figure 2). When examining the staining pattern in each tumor tissue, we found higher expression of TGF-β in peripheral region of tumor tissues, while cen-
Expression level analysis

After quantification of all samples by IHC staining, we found the highest positive rate of PTEN as 85% (17/20) in BPH tissues. With advanced grade of tumor tissues, the expression level of PTEN was significantly decreased, as grade III prostate cancer tissue had PTEN-positive rate at 2.7% (1/37) only (P<0.05, Figure 3A). In contrast to PTEN, TGF-β had significantly lower positive expression rate in BPH tissues at only 10.0% (2/20), while grade III prostate cancer had 86.5% (32/37) positive rate (P<0.05, Figure 3B).

 PTEN mRNA level

We further employed ISH to detect the level of PTEN mRNA. We found similar results as those in IHC staining, as BPH tissues had more intensive staining than cancer tissues (Figure 4). Further quantitative analysis revealed a 90% positive rate (18/20) in BPH tissues, which was remarkable higher than that in cancer tissue (5.4%, 2/37, P<0.05, Figure 5). These results suggested negative correlation between PTEN mRNA contents and advanced grades.

Expression of PTEN and TGF-β and clinical features of prostate cancer

We further collect all results of IHC and ISH staining, and compared them with clinical features of prostate cancer. As shown in Table 1, PTEN expression level was decreased while TGF-β level was increased with advanced histology and clinical stages of prostate cancer. Moreover, expression levels of PTEN and TGF-β were negatively correlated (P<0.01).

Discussion

Current studies have revealed the involvement of PTEN and TGF-β gene in multiple malignant tumors including hepatocyte carcinoma, breast cancer and pulmonary carcinoma [12, 13]. This study investigated the expression of those two genes by both IHC and ISH staining in both nor-
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ormal BPH and prostate cancer tissues across different histological grades and clinical stages, in an attempt to analyze the relationship between gene expression and clinical features. Our results showed 85% positive rate of PTEN expression by IHC staining in BPH tissues, while ISH positive rate was as high as 90%, both of which were significantly higher than those in prostate cancer tissues (13.3% and 21.7%). Moreover, with advanced stages of prostate cancer, the positive rate of PTEN was gradually decreased. TGF-β, on the other hand, had only 10% positive IHC rate in BPH tissues, as contrast to 71.7% in prostate tissues. With advancement of clinical stages, positive rate of TGF-β was further elevated. Our results thus showed a close relationship between expression of PTEN and TGF-β and the malignancy, histological grades and clinical stages of prostate cancer.

As one important tumor-suppressor gene, PTEN exerts its function mainly via inhibiting phosphatidylinositol kinase (PI3K) activity via PI3K/Akt signaling pathway [14]. Under stimulus by growth factors, PI3K can phosphorylate 3,4,5-triphosphate phosphatidylinositol, which acts as the secondary messenger for activating Akt for facilitating cell proliferation and growth [15]. PTEN gene can inhibit PI3K/Akt signaling pathway. It has lipid phosphatase activity and can dephosphorylate 3,4,5-triphosphate phosphatidylinositol, for antagonizing PI3K activity and maintaining Akt activity at a relatively stable level, for maintaining normal metabolism, proliferation, differentiation and apoptosis of cells [16]. Under abnormal expression of PTEN, a cascade reaction may lead to over-activation of Akt, leading to uncontrolled cell proliferation and malignancy transformation. In multiple malignant tumors the PTEN expression disorders have been found [17, 18]. Our results also showed down-regulation of PTEN in prostate cancer, as the lower PTEN expression is always accompanied with higher malignancy.

As one polypeptide cytokine, TGF-β exerts critical functions in both normal and tumor cells. In normal epithelial tissues, it can stimulate cells for differentiation for inhibiting cell proliferation. In tumor tissues, however, it can facilitate cell proliferation and infiltration [8]. This study also observed the expression of TGF-β in normal BPH tissues and prostate cancer with different stages. We found positive relationship between TGF-β positive rate and malignancy, and higher TGF-β expression in peripheral tumor regions, suggesting the possible involvement of TGF-β in the growth, infiltration and invasion of prostate cancer cells.

Our study has shown elevated PTEN expression and decreased TGF-β expression in prostate cancer tissues as compared to BPH tissues, suggesting a negative relationship between PTEN and TGF-β expression. Our results indicated possible correlation between PTEN and TGF-β expression. Previous study has found lowered PTEN mRNA level after over-expressing TGF-β in mice [19]. The treatment of pancreatic carcinoma cell line PANC-1 using TGF-β led to suppressed PTEN expression level [20]. These results agreed that TGF-β might inhibit PTEN expression. One recent study suggested the facilitating of cell growth and infiltration potency in addition to down-regulating PTEN by TGF-β via activating NF-κB [21]. This is consistent with our results.

Currently, novel anti-tumor treatment based on TGF-β and PTEN has become one research hotspot [22]. This study provided evidences for related studies. As the early diagnosis of malignant tumor is one major challenge in clinics, our results regarding correlation between PTEN and TGF-β may provide new insights regarding the early diagnosis of prostate cancer.

Disclosure of conflict of interest

None.

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