

Effect of TRAF6 on Proliferation of Prostate Transitional Zone and Peripheral Zone Cells

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ABSTRACT

The objective of this study was to demonstrate the effect of ubiquitin ligase tumor necrosis factor receptor-associated factor 6 (TRAF6) on promoting proliferation (Pro) of prostate transitional zone (TZ) and peripheral zone (PZ) cells by activating the insulin-like growth factor 1-protein kinase/mammalian target of rapamycin (IGF1-Akt/mTOR) signaling, providing a research basis for benign prostatic hyperplasia (BPH) therapy. In vitro proliferative prostate tissue specimens from patients with BPH were collected and classified into PZ and TZ tissues. A TRAF6 interference lentivirus was utilized to measure the expression levels of IGF1 and TRAF6 in the prostatic tissue matrix. The impact of TRAF6 on cell growth in the matrix of TZ and PZ tissues was analyzed, the TRAF6 level in the matrix of TZ and PZ tissues was explored, and its role in the phosphorylation process under growth factor stimulation was demonstrated. IGF1 and TRAF6 levels in the matrix of TZ tissue were superior to those in PZ tissue. After TRAF6 expression was inhibited, the Pro activity of TZ matrix cells slowed down, while after it was enhanced, the Pro activity of PZ matrix cells was dramatically increased. The TRAF6 in the inhibition group of TZ tissue was greatly lower, while that in the enhancement group of PZ tissue was greatly higher. It was concluded that TRAF6 promotes Pro of prostate TZ and PZ cells by activating the IGF1-Akt/mTOR signaling.

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INTRODUCTION

The prostate is a glandular organ located at the bottom of the male pelvis, responsible for secreting prostatic fluid to supplement semen and maintain normal semen composition and viscosity. Benign prostatic hyperplasia (BPH) is a common prostate disease in which the prostate tissue undergoes hyperplasia, leading to symptoms such as obstructed urine flow (Park *et al.*, 2022; Joseph *et al.*, 2022). Appropriate prostate size and normal function play a critical role in men's sexual health and overall health (Ottaiano *et al.*, 2022; Cao *et al.*, 2022). In clinical practice, BPH is a very common disease that seriously affects men's quality of life (Ling, 2022; Maltese *et al.*, 2017). BPH is more prevalent in men over 50 years of age, and the incidence of BPH is increasing year by year with the increase in aging population (Franco *et al.*, 2022;

Al-Barzinj, 2020). The pathogenesis of BPH is complex and involves multiple factors. Disorders in androgen levels and cell proliferation proliferation are easily associated with the occurrence of the disease (Loffroy *et al.*, 2022; Che *et al.*, 2022).

The mechanism of prostate hyperplasia involves multiple cytokines and growth factor-mediated signaling, such as VEGF and insulin-like growth factor 1 (IGF1) (Enikeev *et al.*, 2022; Gerberding and Golzarian, 2022). Among them, IGF1 is an imperative cytokine that can regulate cell proliferation, metabolism, and other biological processes through the Akt/mTOR signaling. The IGF1-Akt/mTOR signaling acts in cell proliferation, metabolism, and other aspects. Tumor necrosis factor receptor-associated factor 6 (TRAF6) is a key signaling transduction protein in the IGF1-Akt/mTOR signaling, which can activate the pathway and promote the proliferation of the transition zone and peripheral zone (PZ) cells in prostate hyperplasia (Sancer *et al.*, 2022; Trujillo-Rojas *et al.*, 2022). The Akt/mTOR signaling is a very imperative biological signaling, playing a crucial regulatory role in many biological processes. The activity of this pathway is regulated by multiple cytokines and growth factors, of which IGF1 is an imperative one (Wen *et al.*, 2021). IGF1 can promote cell proliferation, metabolism, and other basic biological processes by activating the Akt/mTOR signaling, and has been shown to act in prostate hyperplasia (Li *et al.*, 2022).

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In addition, the Akt/mTOR signaling has been widely studied in many diseases such as diabetes and cancer. In recent years, the regulatory role of TRAF6 in prostate hyperplasia has attracted more and more attention. Some researchers have utilized animal models and cell culture techniques to demonstrate that TRAF6 expression is closely related to the occurrence of prostate hyperplasia and found that overexpression of TRAF6 can promote the proliferation and metabolism of prostate cells (Li *et al.*, 2022). In addition, some experiments that inhibit TRAF6 have shown that blocking TRAF6 can alleviate the pathological state of prostate hyperplasia, providing novel therapeutic options for the treatment of prostate hyperplasia (Khusbu *et al.*, 2020).

The role of TRAF6 in promoting proliferation of TZ and PZ cells in the prostate by activating the IGF1-Akt/mTOR signaling was analyzed in this work, to investigate the role and mechanism of TRAF6 in the progression of prostate enlargement and to provide clinical guidance for the treatment of patients with prostate enlargement.

MATERIALS AND METHODS

In this experiment study, in vitro proliferative prostate tissue specimens from patients with BPH at Shenzhen Longhua District Central Hospital were utilized.

According to the zonal anatomy of hyperplastic prostate tissue proposed by Mycnael, prostate zonal tissue was isolated using seminal vesicle as a marker, and PZ and transition zone (TZ) tissues were dissected for primary cell culture and cell passaging in a culture dish. Lentivirus plasmid construction and transfection were performed using TRAF6 interference lentivirus construction, and SV40-T recombinant plasmid electroporation was utilized to transfect human prostate hyperplasia primary stromal cells. Protein immunoblot analysis and real-time fluorescent quantitative PCR were performed to detect protein expression. CCK8 cell proliferation toxicity assay was performed, and cell viability was determined by measuring the optical density at a wavelength of 450 nm. BPH-1 prostate epithelial cells and primary cultured stromal cells were mixed in a matrix gel and transplanted under the renal capsule. After ten weeks, the tissue volume was visualized under anesthesia.

The following factors were analyzed: (1) IGF1, TRAF6, and SV40-T levels in the stroma of prostate hyperplasia tissue; (2) Impact of TRAF6 in the stroma of TZ and PZ tissues on cell growth at various time points, (3) TRAF6 level, phosphorylation level of Akt, mTOR and P70^{S6K} in the stroma of TZ and PZ tissues in various groups, (4) The volume of mixed tissues of TZ and PZ stromal cells and prostate epithelial cells BPH-1 in various

groups.

Using Excel 2016 to record and summarize the data and SPSS 20.0 for data statistics, $\bar{X} \pm S$ represented metric data and analyzed using *t*-test, while percentages (%) represented count data and analyzed using chi-square test. $P < 0.05$ indicating statistical significance.

RESULTS

Figure 1 presents the levels of TRAF6 in the stroma of TZ and PZ tissue in various groups. TRAF6 expression level in the control group of TZ tissue was 1.00, that in the normal group of TZ tissue was 1.08, and that in the inhibition group of TZ tissue was 0.23. The expression level of TRAF6 in the control group of PZ tissue was 0.52, that in the normal group of PZ tissue was 0.51, and that in the enhancement group of PZ tissue was 0.97. Hence, TRAF6 level in the inhibition group of TZ tissue was markedly lower, and that in the enhancement group of PZ tissue was relatively higher.

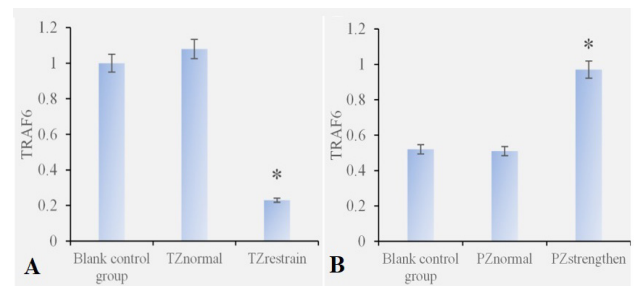


Fig. 1. Different group's TRAF6 levels in TZ and PZ tissue matrices. (A) TZ tissue; (B) PZ tissue.

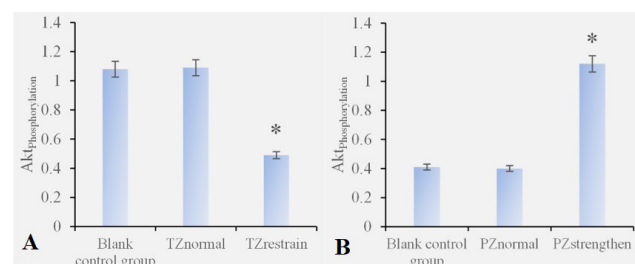


Fig. 2. Akt phosphorylation levels in TZ and PZ matrix of various groups. (A) TZ tissue; (B) PZ tissue.

Figure 2 presents the phosphorylation level of Akt in TZ and PZ tissues with different treatments. The phosphorylation level of Akt in the TZ tissue control group was 1.08, in the TZ tissue normal group was 1.09, and in the TZ tissue inhibition group was 0.49. The phosphorylation level of Akt in the PZ tissue control group

was 0.41, in the PZ tissue normal group was 0.40, and in the PZ tissue enhancement group was 1.12. Therefore, the phosphorylation level of Akt in the TZ tissue inhibition group was relatively lower, and that in the PZ tissue enhancement group was higher.

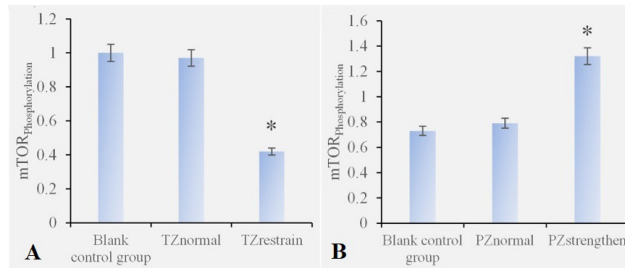


Fig. 3. Differential phosphorylation levels of mTOR in TZ and PZ tissues from various groups. (A) TZ tissue; (B) PZ tissue.

Figure 3 presents the levels of mTOR phosphorylation in the various groups of TZ and PZ matrix. The mTOR phosphorylation level in the TZ tissue control group was 1.00, in the TZ tissue normal group was 0.97, and in the TZ tissue inhibition group was 0.42. The mTOR phosphorylation level in the PZ tissue control group was 0.73, in the PZ tissue normal group was 0.79, and in the PZ tissue enhancement group was 1.32. In summary, the mTOR phosphorylation level in the TZ tissue inhibition group was relatively lower, and in the PZ tissue enhancement group it was higher.

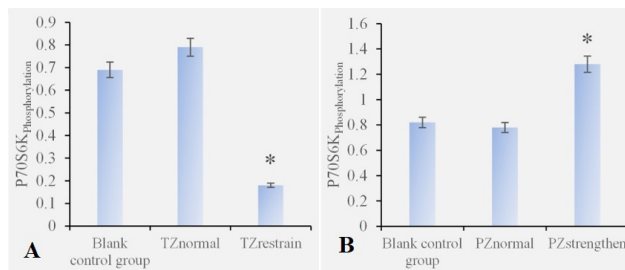


Fig. 4. Phosphorylation levels of P70^{S6K} in matrix of TZ and PZ tissues in various groups. (A) TZ tissue; (B) PZ tissue.

The phosphorylation levels of P70^{S6K} in TZ and PZ tissues with different treatments are shown in Figure 4. The phosphorylation level of P70^{S6K} in the TZ control group was 0.69, the phosphorylation level in the TZ normal group was 0.79, and the phosphorylation level in the TZ inhibition group was 0.18. The phosphorylation level of P70^{S6K} in the PZ control group was 0.82, the phosphorylation level in the PZ normal group was 0.78, and the phosphorylation level in the PZ enhancement group was 1.12.

and the phosphorylation level in the PZ enhancement group was 1.28. Notably, the phosphorylation level of P70^{S6K} was lower in the TZ inhibition group and greatly higher in the PZ enhancement group.

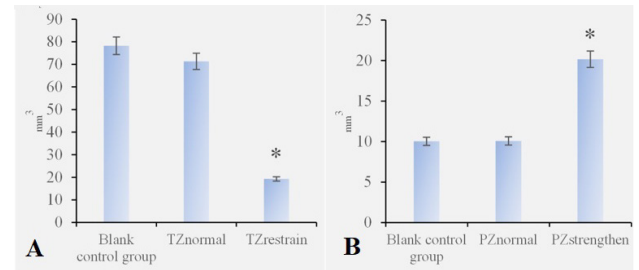


Fig. 5. Volume of mixed culture of TZ and PZ stromal cells with prostate epithelial cell BPH-1 in various groups. (A) TZ tissue; (B) PZ tissue.

Figure 5 presents the volume of the mixture of various groups of TZ and PZ stromal cells with the prostate epithelial cell line BPH-1. The volume of the mixture of stromal cells and BPH-1 cells in the control group of TZ tissue was 78.25 mm³. The volume of the mixture in the normal group of TZ tissue was 71.35 mm³. The volume of the mixture in the inhibitory group of TZ tissue was 19.26 mm³. The volume of the mixture in the control group of PZ tissue was 10.03 mm³. The volume of the mixture in the normal group of PZ tissue was 10.08 mm³. The volume of the mixture in the enhancing group of PZ tissue was 20.15 mm³. Obviously, the volume of the mixture in the inhibitory group of TZ tissue was relatively smaller.

DISCUSSION

BPH is a common male disease characterized by symptoms such as slow urinary flow, frequent urination, and nocturia, which greatly affects men's quality of life (Kusuma *et al.*, 2022; Borziak and Finkelstein, 2022). BPH can easily obstruct the ureter, leading to symptoms such as decreased urine flow rate, urinary retention, and incomplete urination. Long-term BPH can also lead to complications such as infection and stone formation, and may even worsen into prostate cancer, posing a further threat to patients' health. The treatment methodologies for BPH mainly include drug therapy and surgical treatment (Ahani *et al.*, 2022; Huck *et al.*, 2022). Commonly utilized drugs currently include α 1-adrenergic receptor blockers, 5 α -reductase inhibitors, and β 3-adrenergic receptor agonists. TRAF6 is an imperative signal transduction protein in the IGF1-Akt/mTOR signaling, which can respond to cytokine and growth factor signals and regulate the activity of the signaling (Song *et al.*, 2022;

Schagdarsurengin *et al.*, 2022). In BPH, overexpression of TRAF6 can sustain the activation of the IGF1-Akt/mTOR signaling, thereby promoting cell proliferation and metabolism. The regulatory role of TRAF6 is related to the proliferation of BPH cells and regulates the pathological and physiological processes of BPH by participating in the IGF1-Akt/mTOR signaling (Aripaka *et al.*, 2019).

Prostatitis is defined as pathological inflammation of prostate tissue and is one of the most common urological diseases in men. BPH patients experience increased smooth muscle tone and proliferative growth of the prostate, leading to urinary obstruction and urinary symptoms. Studies have noted that various proliferation potentials of matrix cells between the TZ and adjacent regions play an imperative role in prostate hyperplasia, but the molecular mechanisms of this process remain unclear (Hamidi *et al.*, 2017). Shi *et al.* (2018) found, through gene array analysis, that TRAF6 is highly expressed in TZ matrix cells compared to PZ matrix cells. *In vitro* and *in vivo* studies of cell recombination and cell cultures demonstrated that selective downregulation of TRAF6 in TZ matrix cells led to proliferation inhibition, while upregulation of TRAF6 in PZ matrix cells enhanced Proliferation. When TRAF6 was downregulated in primary cultured prostate hyperplasia TZ matrix cells, phosphorylation and ubiquitination of Akt, as well as phosphorylation of mTOR and P70^{S6K}, decreased, proving that TRAF6 can promote proliferation of prostate hyperplasia matrix cells through Akt/mTOR signaling.

In the present study, IGF1, TRAF6, and SV40-T levels in the stroma of prostate hyperplasia tissue, as well as the effects of TRAF6 in the TZ and PZ stroma on cell growth at various time points were analyzed. It also investigated the TRAF6, Akt, mTOR, and P70^{S6K} phosphorylation levels in the stroma of various TZ and PZ tissues, and explored the role of TRAF6 in promoting the proliferation of transition zone and PZ cells in prostate hyperplasia by activating the IGF1-Akt/mTOR signaling. The results showed that the expression level of IGF1 in the matrix of TZ was 2.64, while that in the matrix of PZ was 2.27; the level of TRAF6 in the matrix of TZ was 3.72, and that in the matrix of PZ was 1.97; the level of SV40-T in the matrix of TZ was 0.93, and that in PZ tissue matrix was 0.82. The IGF1, TRAF6, and SV40-T levels in the stroma of TZ tissue were superior to those in the stroma of PZ tissue. On the third day, the OD value of the TZ inhibition group was markedly lower, and the OD value of the PZ enhancement group was higher. Relative to TZ stromal cells with normal TRAF6 expression, cell proliferation was notably slowed down after TRAF6 was suppressed, while relative to PZ stromal cells with normal TRAF6, cell proliferation was enhanced after its expression was increased. TRAF6 level in the TZ tissue

inhibition group was dramatically lower, while that in the PZ tissue enhancement group was considerably higher. The phosphorylation of Akt in the TZ tissue inhibition group was greatly low, while that in the PZ tissue enhancement group was high. The phosphorylation of mTOR in the TZ tissue inhibition group was low, while that in the PZ tissue enhancement group was high. The P70^{S6K} phosphorylation level was low in the TZ tissue inhibition group and high in the PZ tissue enhancement group. As an E3 ligase, TRAF6 acts in the phosphorylation process stimulated by growth factors. The mixture volume of stromal cells and prostatic epithelial cells BPH-1 in the TZ tissue inhibition group was small, and the mixture volume of stromal cells and prostatic epithelial cells BPH-1 in the PZ tissue enhancement group was significantly large. This indicates that TRAF6 is crucial in the regulation of the proliferation of prostatic stromal cells in patients with BPH.

This work has a high clinical adoption value and positive significance. Nevertheless, the limitations of this work are the small sample size, which requires further supplementary research and validation.

CONCLUSION

TRAF6 can promote the proliferation of transition zone and PZ cells in prostate hyperplasia by activating the IGF1-Akt/mTOR signaling. TRAF6 expression inhibition leads to a marked decrease in cell proliferation in TZ matrix, while its enhancement results in a great increase in cell proliferation in PZ matrix. Thus, TRAF6 plays a major role in growth factor-stimulated phosphorylation processes.

Funding

Not applicable.

IRB approval

This study was approved by the Advanced Studies Research Board of Shenzhen Longhua District Central Hospital, Shenzhen, Guangdong Province, 518000, China.

Ethical approval

The study was carried out in compliance with guidelines issued by Ethical Review Board Committee of Shenzhen Longhua District Central Hospital, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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