Apoptotic effects of *Staphylococcus aureus* alpha toxin on DU145 cancereous cells

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ABSTRACT

Prostate cancer is one of the most common cancers among men, which may also affect their fertility. Usually, prostate cancer grows slowly and is initially limited to the prostate gland, but if it grows, it spreads and affects other organs as well. While some prostate cancers are benign and can be cured with minimal treatment, others are malignant and can spread quickly. In this study, the apoptotic effects of Staphylococcus aureus alpha toxin as a recombinant vector on DU145 prostate cancer cells have been investigated.

After culture, the cells were transfected with empty vector and recombinant vector containing Staphylococcus aureus alpha toxin gene using Lipofectamine.After 72 hours, the expression level of apoptotic genes including P53, BCL2 and BAX in normal cells, cells with empty vector and cells with recombinant vector were investigated by QRT-PCR.

The findings showed that the expression level of the mentioned apoptotic genes in cells transfected with the recombinant vector containing Staphylococcus aureus alpha toxin gene had changed significantly and apoptosis had occurred in these cells.

It seems that Staphylococcus aureus alpha toxin has anti-apoptotic and anti-cancer properties on DU145 prostate cancer cells.

Keywords: *Staphylococcus aureus alpha toxin, prostate cancer, P53, apoptotic genes*

1. INTRODUCTION

Prostate cancer is one of the most common cancers among men, which may also affect their fertility. Usually, prostate cancer grows slowly and is initially limited to the prostate gland, but when grows, it can spread and involve other organs [1]. While some prostate cancers are limited in beginning and can be cured with minimal treatment, some are more malignant and can spread rapidly [2]. Nowadays, treatments such as chemotherapy, hormone therapy and radiation therapy are common treatments for this cancer [3]. One of the types of prostate cancerous cells are DU145 cells. DU145 has moderate metastatic potential compared to PC3 cell which has a high metastatic potential. DU145 cell line is derived from central nervous system metastasis, with the primary origin of prostate adenocarcinoma [4].

Bacterial toxins are the first compounds known to cause bacterial diseases in humans and animals. Extensive studies have been conducted to identify bacterial toxins and declare their mechanism of action [5]. Bacterial toxins have been used as carriers of tumor killing agents. Bacterial toxins that have the ability to bind to tumor surface antigens, have been used in the treatment of cancers. Some studies have shown that treated mice with some strains of *Staphylococcus* spp. and *Escherichia coli* that express cytolysin A, inhibits the growth of some tumors [6 and 7].

Staphylococcus aureus secretes a number of destructive toxins in the host's body, one of the most prominent of which is alpha hemolysin (toxin). Recently, ADAM10 has been identified as a cell receptor for alpha toxin. Molecular studies show that this toxin creates pores in epithelial cells membranes and causes host cells lysis. This toxin is able to attach to and oligomerize the heptameric structures in the cell membrane and create the flow of ions such as Ca^{2+} and K^{+} [8]. In this study, the anticancer and apoptotic effects of *Staphylococcus aureus* alpha toxin on prostate cancer cells has been investigated.

2. MATERIALS AND METHODS

2.1 Preparation of Recombinant Vector

The sequence alpha toxin gene was extracted from NCBI database and the pcDNA3.1 vector containing the gene was ordered from the manufacturing company. After checking the correction of enzymatic digestion results by the manufacturing company, the vector map, the recombinant vector, and the simple plasmid were sent to the investigators.

2.2 Vector Extraction

Recombinant vector and empty plasmid were introduced into Top10 *Escherichia coli* utilizing heat shock. After spreading, the bacteria on LB agar medium containing the antibiotic ampicillin, the bacteria containing recombinant vector and the the bacteria containing empty vector were able to grow on this medium. In this step, FAVORGEN kit was used for plasmid extraction. For this purpose, culture medium containing bacteria was sedimented at 1200 rpm and vector extraction was done. In order to determine the quantity of plasmid extraction, the plasmid concentration was determined with a nanodrop device, and to determine its quality, about 2 µlof the extracted plasmid was run on agarose gel electrophoresis.

2.3 Cell Culture and Transfer of Recombinant Vector and Gene-Free Plasmid to Cells

The cells purchased from the Pasteur Cell Center in Iran and were transferred into 75 cm square flasks. Then, RPMI medium mixed with FBS (10%) and was added to the cells and kept in a carbon dioxide incubator for 24 hours. The cells were divided into three groups of treatment, normal and control and transferred to 6-well plates. Then the plasmid and lipofectamine solution were mixed together for 11 minutes and added to the treatment well. The same operation was performed on the cells carring plasmid without gene and added to the control well. Then the plate was placed in a carbon dioxide incubator for 4 to 6 hours.

2.4 RNA Extraction and cDNA synthetisis

In order to investigate the expression of genes effective in the process of apoptosis, following the transfer of vectors to cells, RNA extraction was performed with the RNA plus kit.by adding one µl of DNase enzyme and 2 µl of 10X buffer in order to remove DNA. cDNA synthesis was performed according to the instructions of the Yekta Azma company kit by mixing 1 µl of the extracted RNA from the previous step with µl of oligo dt primer in a nuclease-free microtube and adjusting to a volume of 13.4 µl with DEPC water. The procedure was performed at 47 °C for 60 minutes.

2.5 qRT-PCR to Investigate Apoptotic Genes

qRT-PCR method was used to check the expression level of apoptotic genes including *BAX*, *P53*, and *BCL2* in normal cells and the cells containing recombinant and empty vectors. The characteristics of the primers used in this study are listed in Table 1.

Table 1. *Primers used in this study*

Gene	Sequence	Product size (bp)
a-toxin	ATGGTGCCCTTTGTGCGAATC ACACTGCTGCTGGGCTCCATT	227
P53	TGCGTGTGGAGTATTTGGATGAC CAGTGTGATGATGGTGAGGATGG	170
BAX	AGGTCTTTTTCCGAGTGGCAGC GCGTCCCAAAGTAGGAGAGGAG	154
BCL2	GACGACTTCTCCCGCCGCTAC CGGTTCAGGTACTCAGTCATCCAC	245
GAPDH	GCCAAAAGGGTCATCATCTCTGC GGTCACGAGTCCTTCCACGATAC	183

3. RESULTS

3.1 Vector Map and Location of Alpha Toxin Gene

The manufacturing company performed enzymatic digestion with BamH1 and EcoRV enzymes. The size of the

vector was detected as 5389 bp and the size of the alpha toxin gene was detected as 963 bp (Figure 1).

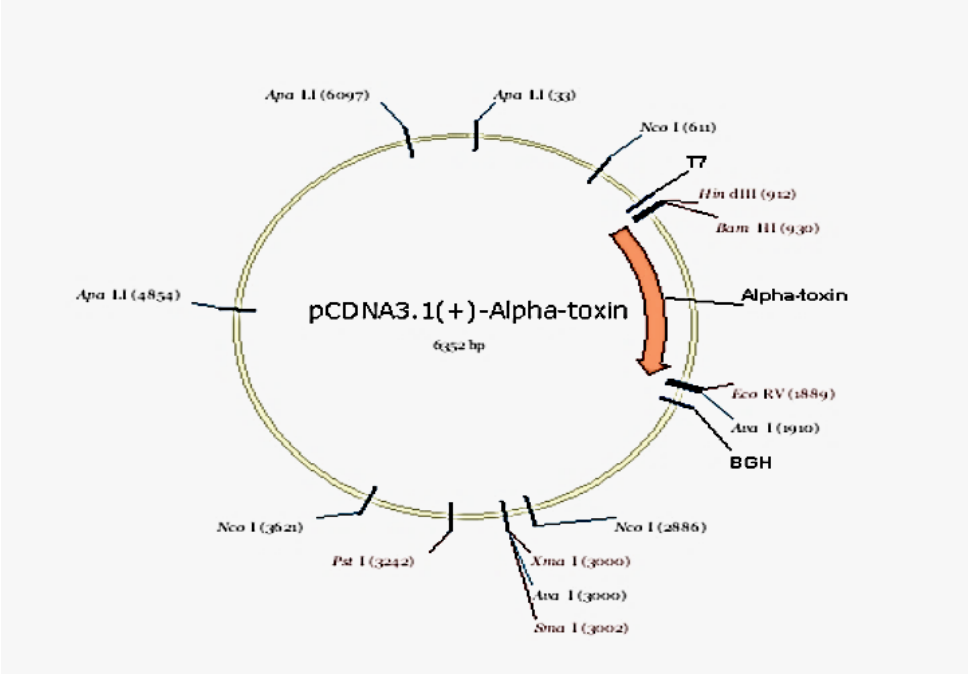

Fig. 1. *The image representing the map of the recombinant vector containing the alpha toxin gene*

3.2 Confirming the presence of alpha toxin gene by PCR method

PCR was performed with specific primers of the alpha toxin gene and its product was detected on agarose gel electrophoresis. The desired 227 bp band was observed on the gel (Figure 2).

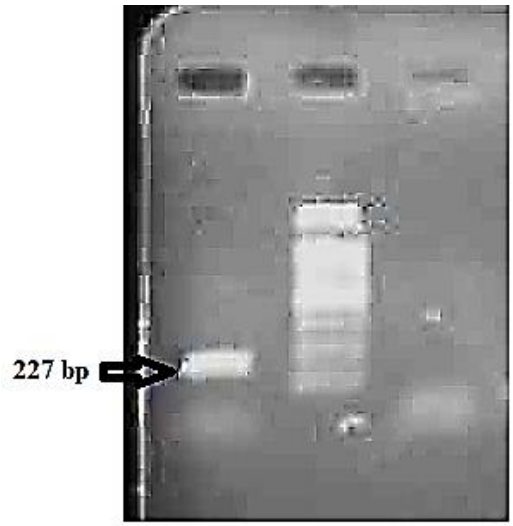

Fig. 2. *The 227 bp band related to alpha toxin according toa 100 bp DNA Ladder*

3.3 View of cultured cells under a light microscope

DU145 cells were separated from the bottom of the flask using trypsin and their morphology was examined under a microscope (Figure 3).

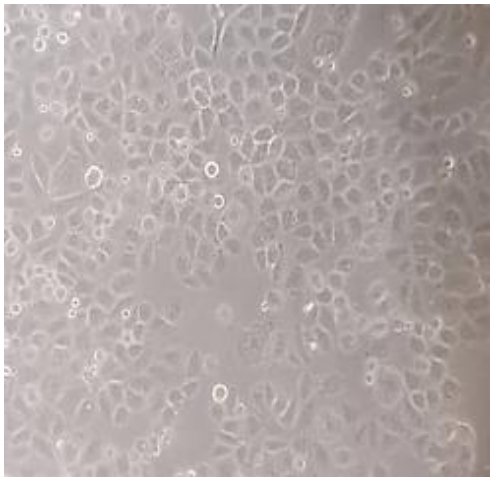

Fig. 3. *DU145 cells under light microscope at 40 magnification*

3.4 The results of statistical analysis on the expression level of apoptotic genes calculated by QRT-PCR
The studied groups were 3 groups including cells with recombinant vector containing alpha toxin gene, plasmid without gene and normal cells. Comparison of gene expression was done using Rotor-Gene Real-time analysisb software based on Livak method. The obtained data were analyzed by SPSS software and ANOVA test (Figure 4, Figure 5 and Figure 6). The results shown in Figure 4 indicates that the expression level of *BCL2* gene in cells containing recombinant vector has decreased significantly ($p<0.05$) compared to cells containing normal plasmid without gene.

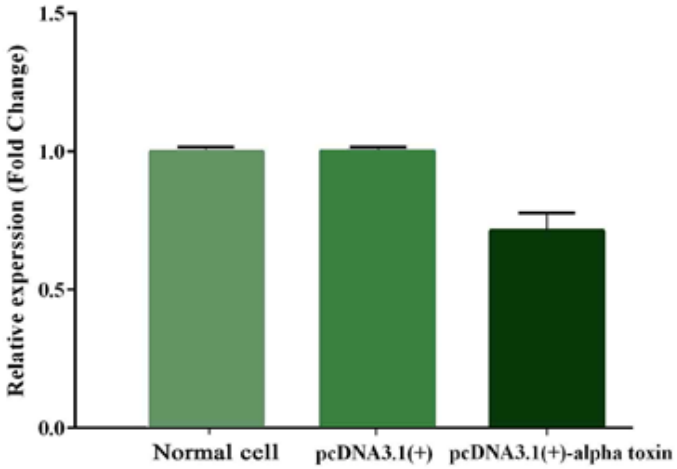

Fig. 4. *BCL2* genes expression diagram

The results shown in Figure 5 indicates that the expression level of *BAX* gene in cells containing recombinant vector with alpha toxin gene is significantly increased compared to normal cells and cells containing plasmid without gene ($p<0.05$).

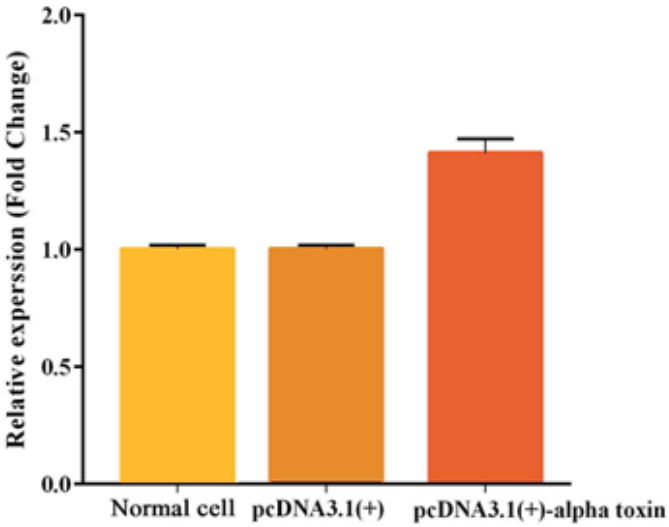

Fig. 5. *BAX* genes expression diagram

The results shown in Figure 6 indicates that the expression level of p53 gene in cells with recombinant vector containing alpha toxin gene is significantly increased compared to cells with plasmid without gene and normal cells ($p<0.05$).

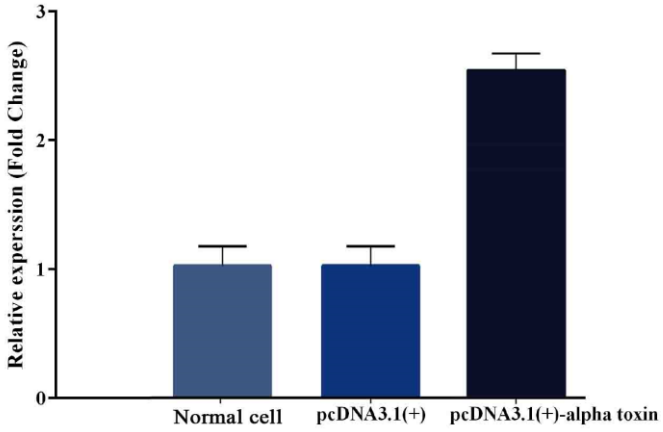

Fig. 6. *P53* genes expression diagram

4. Disscussion

Prostate is a small gland located under the bladder in the upper part of men urethra. In developed countries, prostate cancer is the second most common cancer (after skin cancer) and the second deadliest cancer (after lung cancer) in men [9]. One out of every six men gets this cancer. This cancer is the most common male malignancy and the second or third cause of death among western men. Prostate gland age-related growth is a non-proliferative diseases. Normal, ranging from benign hyperplasia to obvious malignancies have been identified for this gland [10]. Proposed treatments for prostate cancer, such as chemotherapy and radiotherapy, cause apoptosis in normal cells, so new treatments that cause apoptosis only in cancer cells are of interest, and toxin therapy is one of these methods [11]. Alpha toxin of *Staphylococcus aureus* is one of the toxins with anti-cancer properties, which can cause apoptosis by creating pores in the host cell and cause the death of cancer cells [12]. In this study, the apoptotic effects of *Staphylococcus aureus* alpha toxin was investigated on prostate cancer cell, DU145. For this purpose, the recombinant vector containing the gene of this toxin was designed and inserted into TOP10 *Escherichia coli* for proloferation and then the plasmid was extracted. Then, the recombinant and empty vectors were introduced into the cultured cancer cells separately using lipofectamine and finally, after RNA extraction, cDNA was synthesized and real time PCR was used to express the toxin gene and apoptotic genes by using *GAPDH* as a reference gene. The cancer cell line was investigated and they were compared with the same data in

untreated and untransformed cells. After statistical analysis, it was found that the expression level of *P53* and *BAX* genes in cells containing recombinant vector increased significantly compared to other cells ($p < 0.05$). Also, the level of *BCL2* gene expression in these cells was significantly reduced compared to other cells ($p<0.05$).

In a similar study by Zhang et al. (2017), different effects of *Staphylococcus aureus* toxins on the expression of genes such as *BAX*, *FAS* and *BCL2* in PC3 cells were investigated and it was found that alpha toxin is able to change the expression of these genes and cause apoptosis. The results of this research are consistent with the results of our study and indicate the anti-cancer effects of this toxin [13]. In a study in 2019, the recombined plasmid pcDNA3.1 (+)-seb was introduced into AGS cells by lipofection method after replication in AGS cells. After 10 days of treatment with neomycin antibiotic, total cell RNA was extracted and cDNA was synthetized for performing real time PCR on apoptosis genes *p53*, *BAX*, *caspase 3*, and *Bcl-2* as well as glyceraldehyde. The results showed a significant increase in the expression of *BAX* and *p53* genes and a significant decrease was seen in the expression of Bcl-2 and caspase 3 genes. Compared to AGS cells that did not receive the *seb* gene, the cells containing *seb* gene progressed more towards apoptosis. According to the findings of that study, it can be concluded that enterotoxin B expressed in gastric cancer cells increases the expression of pro-apoptotic genes and decreases the expression of anti-apoptotic genes. According to evidence, this toxin can act as an anticancer agent in AGS cell line. Similar to our study, they showed the anti-cancer effects of some bacterial (*Staphylococcus aureus*) toxins [14]. In a study in 2023, the recombinant pBudCE4.1-cpe vector and the empty plasmid were separately transfected into DU145 cell line using Lipofectamine 2000 protocol. The presence of each vector was checked by PCR. The *cpe* gene expression was investigated in transfected DU145. Expression of apoptosis genes (*FAS*, *BCL2*, *BAK* and *BAX*) and cell cycle progression genes (*cyclin E* and *cyclin D1*) were maintained in transfected and non-transfected DU145 cells. Statistical analysis showed that the expression of *BAK*, *BAX*, and *FAS* genes was significantly higher in cells transfected with a recombinant vector ($P < 0.05$). The expression of *BCL2*, *cyclin E* and *cyclin D1*genes in DU145 cells transfected with vector was significantly decreased compared to cells transfected by empty plasmid or non-transfected cells. Expression of *cpe* can suppress DU145 growth by affecting cell apoptosis. The expression of *cpe* in the DU145 cell line was tested for the first time, confirming its possible effect on similar cells.The results of this study, similar to our study, show the anti-cancer effects of a number of bacterial toxins in the form of recombinant vectors, including prostate cancer [15].

5. Conclusion

According to our findings, *Staphylococcus aureus* alpha toxin as a recombinant vector has anti-cancer and apoptotic properties and is a new approach in the treatment of some cancers, including prostate cancer.

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