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Effects of *Staphylococcus aureus* enterotoxin type A on inducing the apoptosis in cervical cancer cell line☆,☆☆

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ABSTRACT

Recently, a number of studies have revealed that biotoxins present great potential as antitumor agents, such as some bacteria toxins, so they could be used as chemotherapeutic agents against tumors. In this study, the effects of *Staphylococcus aureus* enterotoxin type A on BAK, FAS, BAX, TNF-α, p53, BCL-2 and Survivin genes expression in HeLa cell lines were investigated. These cells are transfected with the pcDNA3.1(+)-sea (recombinant) and pcDNA3.1(+) (non-recombinant) plasmids. The expression of BAK, FAS, BAX, TNF-α, p53, BCL-2 and Survivin genes in transfected cells were then analyzed by real time PCR. The results showed an increase in the expression of BAK, FAS, BAX, TNF-α and p53 genes and decreased BCL-2 and Survivin expression at a significant level. As compared to the HeLa cell, which did not receive the sea gene, the cells containing the toxin gene had progressed more towards apoptosis. *Staphylococcal* enterotoxin type A has an inhibitory effect on the growth, proliferation and invasion of breast and cervical cancerous cells through altering the expression of the genes involved in the apoptosis process. Therefore, it seems that there is a good research field for the use of this toxin in the control and treatment of such malignancies.

1. Introduction

Cervical cancer is the most common gynecological malignant tumor and the second most prevalent malignant tumor among women after breast cancer (Schiffman and Solomon, 2013). Every year, more than half a million women are diagnosed with cervical cancer, which causes 300,000 deaths worldwide (Cohen et al., 2019). Furthermore, ~569,847 new cases of cervical cancer were diagnosed and 311,365 cervical cancer related-mortalities cases occurred worldwide in 2018 (Bray et al., 2018). In addition, cervical cancer remains the third most common type of cancer in developing countries (Tsikouras et al., 2016).

Bacterial toxins play an important role in the pathogenesis of these infectious agents, and many bacteria are able to produce toxins, extracellular enzymes, and pigments (Allison et al., 2014; Ramachandran, 2014). Although bacterial toxins appear to be harmful to humans and other hosts, researchers are now finding useful applications for these biological products. The role of bacteria as anti-cancer agents has been known for many years (Sabzehali et al., 2017). A number of bacterial toxins such as *S. aureus* alpha-toxin (α-toxin), Clostridium Perfringes Enterotoxin (CPE), and streptolysin O (SLO) of *Streptococcus pyogenes* are most attractive for their application in cancer therapy (Forbes, 2010). Bacterial toxins are the most obvious cytotoxic agents because these genes are native to bacterial physiology. Cytolysin A (HlyE) is a toxin that acts by making pores in the mammalian cell membranes and inducing apoptosis. Apoptosis induced by bacterial toxins during infection is common and is now regarded as a critical function in disease processes (Pahle and Walther, 2016).

Staphylococcus aureus is a gram-positive, non-spore-forming, capsule-free, immobile, aerobic or anaerobic bacterium. *S. aureus* is the second most common cause of food poisoning after the Salmonella. Within a few

Abbreviations: Sea, *Staphylococcus aureus* enterotoxin type A; PCR, Polymerase Chain Reaction; Bcl-2, B-cell lymphoma 2; BAX, Bcl-2-associated X protein; TNF-α, Tumor necrosis factor alpha; BAK, BCL2 antagonist/killer; WHO, World Health Organization; DMEM, Dulbecco's Modified Eagle Medium.

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hours, the organisms in the jejunum produce the emetic enterotoxins that induce vomiting and secretion of water and electrolytes into the bowel as well as immune stimulation. These intermediate-sized polypeptides (28–34,000 MW) cross the epithelial cell membranes and enter the paracellular space where the Intraepithelial Lymphocytes (IEL) reside (Roberts et al., 2000). The host immune system may conceivably enable *S. aureus* infection at the time of apoptosis, and this process in tissue cells can also stimulate the immune response through cytokine production and T cell differentiation. Therefore, the pathogenesis *S. aureus* can be influenced by the apoptosis (Torchinsky et al., 2010). Several toxins of *S. aureus* such as Staphylococcal Enterotoxins (SEs) and α-toxin have proapoptotic activities. SEs include at least 10 members, namely SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SER, and SET, which can cause food poisoning in some cases (Pearson and Murphy, 2017).

Alpha toxins are water-soluble secretory monomers with a molecular weight of 33.3 kDa that, upon attachment to the cell, oligomerize and form heptamer pores in the plasma membrane of the host cell, and like enterotoxins in different cells apoptosis. They induce initiators from different pathways such as internal path, external path and even caspase pathway apoptosis (Iacovache et al., 2010; Srivastava et al., 2009).

The Bcl-2 family of proteins is completely related to the regulation of apoptosis. While Bcl-2 and Bcl-x show remarkable inhibitors of apoptosis, the BAX subfamily such as BAX, Bak and the BH3-only subfamily induces programmed cell death (Hsu et al., 1995). It is also true for the apoptosis induced by cytotoxic treatment (Kale et al., 2018). From the members of the Bcl2 family, BAX has been suggested to be a key regulator of the apoptotic machinery, transcriptionally activated by upstream molecules, such as the tumor suppressor p53 (Toshiyuki and Reed, 1995). However, BAX-induced apoptosis through p53 independent mechanisms in response to anticancer agents has been detected in colorectal cell line investigations as well (Park et al., 2012). The p53 plays a key role in cell cycle arrest and initiation of apoptosis. This is a tumor suppressor gene which is inactivated in the development of many malignancies including gastric cancer. The expression rate of p53 determined by immunohistochemistry has been reported as 13–54% in gastric cancer (Karim, 2014). A cell without mutation does not represent immunohistochemical staining of p53 because there is no such growth in the cell (Pietrantonio et al., 2013). Prognostic role of p53 expression in gastric cancer has been investigated in many studies, Some of them have suggested that the patients without p53 expression have longer survival because non-functional p53 expression in GC tissues was usually related to more lymph node metastasis and a poor prognosis (Ye et al., 2012). Reverse to these studies, some reports have presented that p53 expression is not associated with survival and other clinical-pathological parameters (Tsujitani et al., 2012).

On the other hand, it has been proposed that the regulation of the cellular suicide program in response to cellular stress depends on the dynamic collaboration between Bcl-2 and BAX. As a consequence, higher related BAX levels might result in apoptotic cell death, whereas higher related Bcl-2 levels might inhibit this program in response to genotoxic stress (Olatavi et al., 1994). An emerging family of Ced-3/Ice-like cysteine proteases (caspases) has been also recognized, and numerous studies have demonstrated their contribution in executing the process of cell death itself. There are three subfamilies of caspases based on their degrees of homology and by phylogenetic analysis; the ICE-related family includes Ice/caspase-1 itself with its spliced isoforms, TX/ICH-2/ICE rel-II/caspase-4, TY/ ICE-rel-III/caspase-5, and ICH-3/caspase-11; the CPP32-related family includes CPP32/Yama/Apopain/caspase-3, Mch-2/caspase-6, Mch3/ICE LAP3/CMH-1/caspase-7, Mch-4/caspase-10, and Mch 5/FLICE/MACH/caspase-8, and the third group includes ICH-1/caspase-2, and ICE-LAP6/Mch6/caspase-9 (Smith and Bayles, 2006). Consequently, the present research carried out to investigate the expression of BAX, p53, caspase3, and Bcl-2 genes in AGS cell line transfected with a recombinant vector containing a gene that encodes the Staphylococcal Enterotoxin type B (SEB). To increase our knowledge of the inhibition process of anti-apoptosis genes, we selected p53, Caspase3, BAX, Bcl-2 genes for evaluation of *Staphylococcus aureus* enterotoxin B effects in cancer cells (Ghaleh et al., 2019).

Because of the limitations in cancers treatment, the ability of cancer cells in metastatis, and the lack of definitive treatment in advanced cases, the research into finding ways to control this deadly disease seems necessary. Due to the large evidence of bacterial toxins' role in controlling cancers from different pathways, in this study, we try to evaluate the Effects of genes encoding the enterotoxin toxins *Staphylococcus aureus* on the expression of apoptotic genes in cancer cells.

2. Materials and methods

2.1. Preparation of recombinant vector

The sequences of *S. aureus* sea gene in order to expression in mammalian cells (human HeLa cells) were obtained from GenBank (ACCESSION: EF520720). Then, the gene optimized by the script online software was examined. Via Generay Biotech Co., Ltd. (Shanghai, China), the pcDNA3.1(+)-*sea* recombinant vector was constructed. Heat shock method was used to transform the recombinant plasmid pcDNA3.1(+) and empty plasmid (Invitrogen) into $CaCl_2$ chemically competent *E. coli* TOP10F cells (Hashemzehi et al., 2018). The transformed bacteria were plated on Luria-Bertani (LB) medium containing 100 μg/ml ampicillin. The extraction of plasmids was done by the Favorgen plasmid DNA Extraction mini kit according to the manufacturer's protocol. The pcDNA3.1(+)-*sea* was confirmed by *BamHI/EcoRV* enzymatic double digestion and PCR.

2.2. Cell culture and transfection

HeLa cell lines were purchased from National Cell Bank of Iran (NCBI, Pasture Institute of Iran, Tehran). HeLa cell in a T-75 flask with DMEM and DMEM-F12, culture medium, respectively (Dulbecco's Modified Eagle's medium) (Sigma-Aldrich, St., MO, USA), supplemented with 10% fetal bovine serum (Sigma-Aldrich, St., MO, USA) and 1% Penicillin-Streptomycin (Gibco BRL, Karlsruhe, Germany), were cultured and incubated at 37 °C in an environment with 5% CO_2 and 90% humidity. The medium was changed three times a week and trypsin/EDTA (Invitrogen, USA) was used to remove the cells. In order to transfect HeLa cells, they were cultured as mentioned above. When the confluence of cells reached 85%, the HeLa separately were seeded in three wells of 6-well tissue culture plates (1×10^6 cells/well). Then, by use of lipofectamine 2000 reagent (Invitrogen, USA), HeLa cells in the first and second wells of the plates were transformed by pcDNA3.1(+)-*sea* (as recombinant plasmid), and empty pcDNA3.1(+) (as control plasmid), respectively according to the manufacturer's instructions. Each transfected well was supplemented with 4 μg plasmid per each well [plasmid (μg): Lipofectamine 2000 (μl) = 1:3], in a serum-free medium. Four hours following transfection, the media were replaced with fresh medium containing 600 mg/l G418 and 10% FBS for 10 days. To accurately assess the performance of G418, there was no transfected cell in the third well. Ultimately stably transfected clones of both cell lines were selected by G418 screening. These clones were shown to express mRNA from the introduced plasmid. After transformation, the frequency of transfected cell was $>1 \times 10^6$ cells used initially.

2.3. RNA extraction and cDNA synthesis

Using RNX-Plus reagent (SinaClon, Iran), total RNA was separately extracted from both transformed and untransformed HeLa cells according to the manufacturer's instructions. The concentration and purity of the extracted RNAs were evaluated by Nano drop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), read at wave lengths of 230 nm, 260 nm and 280 nm. Subsequently, all RNA sequences were converted cDNA and transferred to −20 °C through a cDNA synthesis kit (RevertAID First Standard cDNA Syn Kit), (Termo

Scientific, Lithuania).

2.4. RT-PCR reaction and dot blotting

To determine the expression of the *sea* gene in transfected HeLa, RT-PCR reaction mixture with a final volume of 25 μl containing 3 μl of cDNA, 2.5 μl of 10× buffer, 0.75 μl of 50 mM $mgcl_2$, 0/5 μl 10 μM dNTP, 0/5 μl Taq DNA polymerase, 1 μl of each primer (10 pmol/ml) and 15/75 μl ddH_2O, was ready and the reaction within the Thermal Cycler in 30 cycles carried out. According to the program, initial denaturation for 5 min at 94 °C, denaturation for 1 min at 94 °C, annealing for 60 s at 56 °C and final extension for 5 min at 72 °C was carried out. Finally, electrophoresis was performed on 1% agarose gel for PCR product evaluation. Dot blot method is used to confirm the specificity of the *sea* gene expression. Briefly, for this purpose, 8 μL of proteins extracted from HeLa cells transformed with recombinant plasmid (pcDNA3.1 (+)-sea) was dotted on nitrocellulose membrane (Millipore, USA). Non-specific binding sites were then blocked using TBS-T (Merck, Germany) for 30 min at room temperature. The membrane was washed three times with TBST, incubated for 30 min with anti-histag HRP-conjugated monoclonal antibody (Sigma, USA) with a dilution of 1:1000. Following three washes with PBS buffer, the substrate was used for the detection. The PBS was utilized as a technical negative control in experiment.

2.5. Real time RT-PCR

The expression of BAK, BAX, BCL2, FAS, p53, Survivin and TNF-α genes was analyzed by real time PCR (Rotor gene 6000 corbett, Australia) using DNA Master SYBR Green 1 (Roche Diagnostics GmbH Mannheim, Germany). A PCR reaction mixture containing 5 mM of $MgCl_2$, 2 μl of SYBR Green 1 mix, 2–4 μl of cDNA and a pair of primers. Seven pairs of primers were separately used: six pair to amplify the BAK, BAX, BCL2, FAS, p53, Survivin and TNF-α genes, the other pair for the endogenous control gene, GAPDH. All samples were amplified in a 20 μl PCR reaction containing specific primers (Table 1), GAPDH gene and *SYBR Green* as an internal control and reporter, respectively. No template controls (NTC) were included in each run. Real-time runs were performed on the Corbett-Research Rotor Gene 6000 (Sydney, Australia). The amplification program consisted of 1 cycle of 95 °C with a 4 min hold, followed by 40 cycles of 95 °C with a 15 s hold, annealing temperature at 64 °C (for FAS, p53, Survivin and GAPDH), 65 °C (BAX, BCL-2 and TNF-α) and 66 °C (BAK) with a 20 s hold, and 72 °C with a 20 s hold. Following amplification, a melting curve was run to confirm that a single PCR product was amplified. Relative quantification analysis was performed by using the comparative C_T method $(2^{-\Delta\Delta Ct})^{20}$. To verify the product sizes, the PCR products were electrophoresed on 2% agarose gel.

2.6. Statistical analysis

All data were presented as mean ± SD. Paired Student *t*-test was performed for statistical analysis. The level of statistical significance was set at $p < 0.05$ (Table 2).

3. Results

3.1. RT-PCR analysis and expression of sea mRNA

To confirm the mRNA expression of *sea* gene encoding staphylococcal enterotoxin type A, HeLa cells which were transfected with both recombinant plasmid (pcDNA3.1(+)-*sea*) and empty plasmid (pcDNA3.1 (+)) were examined. The result show that the 203 bp fragment related to the *sea* cDNA, amplified by RT-PCR only in HeLa cells transformed with recombinant plasmid (pcDNA3.1(+)-*sea*) (Fig. 1). In the following, as shown, the extracted protein content appeared as a light stain indicating the binding of specific anti-his-tag monoclonal antibody interaction with the expressed protein of *sea* gene (Fig. 2).

3.2. RT-real time PCR

Further examined was the expression of BAK, BAX, BCL2, FAS, p53, Survivin and TNF-α genes in HeLa cells transformed by recombinant plasmid (pcDNA3.1(+)-*sea*) and empty plasmid (pcDNA3.1(+)), as

Table 2
BAX, BAK, BCL.2, FAS, Survivin and TNF-a mRNA expression in transformed HeLa cells by pcDNA3.1(+)-sea.

Cell	Parameters		Mean	Std.D	*p*-Value
HeLa	BAK	PcDNA3.1(+)	1.003179	0.112849	0.0049**
		PcDNA3.1(+)-sea	2.266108	0.055529	
	BAX	PcDNA3.1(+)	1.000865	0.058833	0.0118*
		PcDNA3.1(+)-sea	2.037710	0.149676	
	BCL.2	PcDNA3.1(+)	1.001302	0.073482	0.0453*
		PcDNA3.1(+)-sea	0.760562	0.014910	
	FAS	PcDNA3.1(+)	1.000385	0.039215	0.0174*
		PcDNA3.1(+)-sea	2.011296	0.187031	
	Survivin	PcDNA3.1(+)	1.390345	0.047666	0.0081**
		PcDNA3.1(+)-sea	0.772109	0.056771	
	TNF-α	PcDNA3.1(+)	1.001737	0.083370	0.0302*
		PcDNA3.1(+)-sea	1.506925	0.096154	
	P53	PcDNA3.1(+)	0.817167	0.050723	0.0296*
		PcDNA3.1(+)-sea	1.390153	0.018639	

Table 1
Forward and reverse primer sequences for real time RT-PCR.

Genes	Forward and reverse	Sequences	Annealing temperature (°C)	Product length (bp)
Sea	Forward	5′-TATGGTTATCAATGTGCGGGTG-3′	64	203
	Reverse	5′-CTTGAAGATCCAACTCCTGAACAG-3′		
BCL-2	Forward	5′-GACGACTTCTCCCGCCGCTAC-3′	65	245
	Reverse	5′-CGGTTCAGGTACTCAGTCATCCAC-5′		
BAX	Forward	5′-AGGTCTTTTTCCGAGTGGCAGC-3′	65	234
	Reverse	5′-GCGTCCCAAAGTAGGAGAGGAG-3′		
FAS	Forward	5′-CAATTCTGCCATAAGCCCTGTC-3′	64	163
	Reverse	5′-GTCCTTCATCACACAATCTACATCTTC-3′		
BAK	Forward	5′-CGTTTTTTACCGCCATCAGCAG-3′	66	154
	Reverse	5′-ATAGCGTCGGTTGATGTCGTCC-3′		
TNF-a	Forward	5′-GCCTCTTCTCCTTCCTGATCGTG-3′	65	184
	Reverse	5′-TTTGCTACAACATGGGCTACAGG-3′		
Survivin	Forward	5′-AGAACTGGCCCTTCTTGGAGG −3′	64	170
	Reverse	5′-CTTTTTATGTTCCTCTATGGGGTC -3′		
GAPDH	Forward	5′-GCCAAAAGGGTCATCATCTCTGC-3′	64	183
	Reverse	5′-GGTCACGAGTCCTTCCACGATAC-3′		
P53	Forward	5′-TGCGTGTGGAGTATTTGGATGAC-3′	64	170
	Reverse	5′-CAGTGTGATGATGGTGAGGATGG-3′		

Fig. 1. The result of RT-PCR on cells receiving the recombinant vector carrying the SEA gene; (1) RT-PCR with specific primers of SEA gene on cells transfected with vector pcDNA3.1 (+) - SEA showing 203 bp band. (2) Positive control, PCR with specific primers of SEA gene on recombinant vector pcDNA3.1 (+) – SEA; (3) Negative control, RT-PCR with specific primers of SEA gene on cells without plasmid and (4) Marker 100 bp.

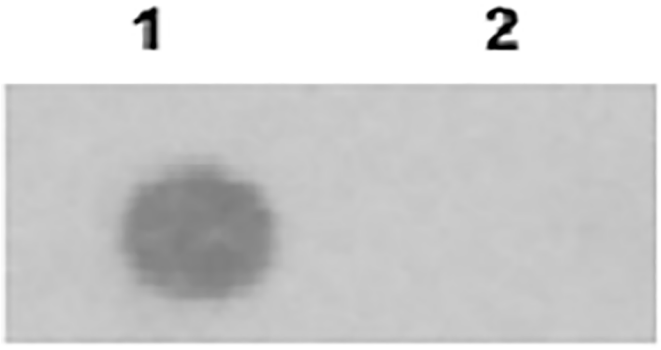

Fig. 2. (1) Result of dot blot analysis (2) negative control [PBS].

control. The melting curve was generated to screen for primer dimmers and to document single product formation for each gene (BCL2, TNF-a, BAX, FAS, p53, Survivin and BAK) in both cell lines. It was drawn based on the temperature (x axis) and dF/dT derivation (y axis). The amplification product of BCL2, TNF-α, BAX, FAS, p53, Survivin and BAK genes was compared to the reference gene product by amplification analysis curve and to demonstrate the efficiency of each PCR reaction, the concentration was plotted against the crossing points (Ct) to create a BCL2, TNF-α, BAX, FAS, p53, and BAK standard curve with $r = -0.99$. Analysis of Real Time PCR Ct values via $2^{-\Delta\Delta ct}$ formula showed a significant decrease in the expression of BCL-2 gene and a significant increase in the expression of TNF-α, BAX, FAS, p53, and BAK genes in *sea*-treated HeLa cell compared to control cells ($p < 0.05$). The results of this study indicated that *staphylococcal* enterotoxin type A remarkably changes the expression of apoptotic related genes in both cell lines. While, the expression of BAK, FAS, BAX, p53, and TNF-α genes, in HeLa cell line were increased significantly, the expression of BCL-2 and *Survivin* genes in HeLa cell markedly reduced in comparison to control group (Fig. 3).

4. Discussion

Cancers remain one of the major challenges of the 21st century. The increasing number of cases is not accompanied by adequate progress in therapy. The standard methods of treatment often do not lead to the expected effects. Therefore, it is extremely important to find out new, more effective treatments (Mo et al., 2012). In recent decades, bacteria's therapeutic role has aroused attention in medicinal and pharmaceutical research. While bacteria are considered among the primary agents for causing cancer, recent research has shown intriguing results suggesting that bacteria can be effective agents for cancer treatment. In order to reduce or prevent endogenous production of antibodies against to the toxin(s) or other unwanted side-effects, human-derived or human-compatible antitoxins as an adjunct to therapy with a combination of toxins can be used. State-of-the-art genetic engineering has been recently applied to bacteria therapy and resulted in a greater efficacy with minimum side effects (Song et al., 2018). The role of bacteria as anticancer agent was recognized almost hundred years ago. The German physicians W. Busch and F. Fehleisen separately observed that certain types of cancers regressed following accidental erysipelas (*Streptococcus pyogenes*) infections that occurred whilst patients were hospitalized. Independently, the American physician, William Coley, noticed that one of his patients suffering from neck cancer began to recover following an infection with erysipelas. He began the first well-documented use of bacteria and their toxins to treat end stage cancers (Zacharski and Sukhatme, 2005). Current studies further indicate that bacterial toxins can control the differentiation, proliferation and apoptosis of the cells (Pahle et al., 2017). For example, reviews in 2003 and 2010 showed that *Salmonella* and *Clostridia* have emerged as agents with high antitumor potential (Leschner and Weiss, 2010; Minton, 2003). In 2012, treating metastatic melanoma with an auxotrophic *Salmonella typhimurium* showed insignificant tumor regression (Zhao et al., 2012). Also understood is that *S. aureus* as a major human pathogen has various toxins like *staphylococcal* enterotoxins which have reportedly triggered apoptosis. This feature turned the old pathogen into a new treatment for human health (Aziz et al., 2014).

Herein, a study was done at the molecular levels to evaluate the effect of the recombinant plasmid on the apoptosis of the HeLa cell line. The pcDNA3.1 (+) mammalian expression vector was used to insert the encoding gene of SEA.

The recombinant plasmid called pcDNA3.1 (+)-sea was constructed and amplified in a TOP10F chemically competent cell.

HeLa cell line was transfected by the pcDNA3.1 (+)-sea and pcDNA3.1 (+) (scramble plasmid) and then, the results of RT-qPCR assay confirmed the positive expression of sea gene in the transfected cells. HeLa cells lipofected by the pcDNA3.1 (+)-sea showed more cell death compared to the controls, which was statistically significant ($P < 0.05$). Therefore, our finding showed the appropriate role of the pcDNA3.1 (+)-sea on cell death. In addition, the expression of BAK, FAS, BAX, TNF-α and p53 genes increased in the sea -treated cells in the HeLa cell line. Also, the findings showed that the expression of Survivin and BCL-2 genes significantly decreased in the HeLa cells transfected with the pcDNA3.1(+)-sea compared to those transfected with empty pcDNA3.1(+) vector ($P < 0.05$).

In the present study, the effects of SEA were investigated on the HeLa cell line, hence; our findings represented the same results regarding the apoptosis induction. These results indicated that *S. aureus* SEA toxin arrests the progression of the cell cycle that leads to activation of the apoptosis through pro-apoptotic genes (BAK, FAS, BAX, TNF-α and p53).

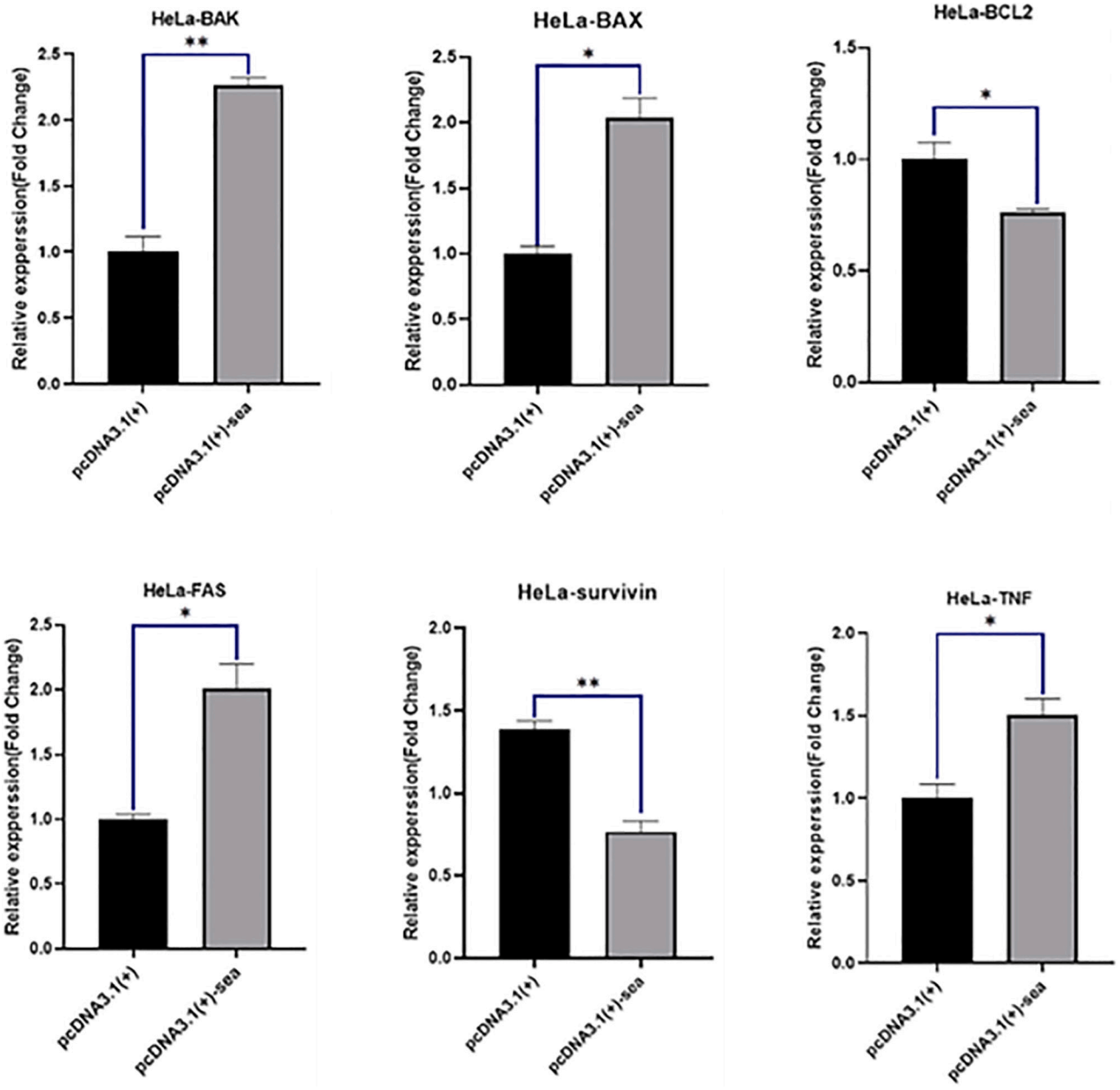

Fig. 3. BAX, BAK, FAS, TNF-α, BCL2, Survivin and p53 mRNA expression in HeLa cell transformed by pcDNA3.1(+)-sea.

5. Conclusion

Resistance to conventional anticancer therapies in patients with advanced solid tumors has prompted the need for alternative cancer therapies. Bacterial super antigen, including *Staphylococcal* enterotoxins are novel and useful agents for killing tumor cells, enhancing antitumor immunity and treating tumors in a tumor-bearing subject. The success of such novel cancer therapies depends on their selectivity for cancer cells with limited toxicity to normal tissues. Further investigation and developments in these studies will add a new dimension to cancer treatment.

CRediT authorship contribution statement

S.A. and A.D. carried out the experiment. Z.N. wrote the manuscript with support from A.K. M.H. and P.K. fabricated the sample. N.B helped supervise the project. A.D. and S.A. conceived the original idea. A.D. supervised the project. A.B. and S.A. designed the model and the computational framework and analyzed the data. Z.N and A.K. carried out the implementation. A.D. performed the calculations. A.D. and S.A. wrote the manuscript with input from all authors. M. H. and N. B. conceived the study and were in charge of overall direction and planning.

Declaration of competing interest

No any conflict of interest.

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References

Allison, Steven D., et al., 2014. Extracellular enzyme production and cheating in Pseudomonas fluorescens depend on diffusion rates. Front. Microbiol. 5, 169. https://doi.org/10.3389/fmicb.2014.00169.

Aziz, M., Jacob, A., Wang, P., 2014. Revisiting caspases in sepsis. Cell Death Dis. 5 (11), e1526. https://doi.org/10.1038/cddis.2014.488.

Bray, Freddie, et al., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 68 (6), 394–424. https://doi.org/10.3322/caac.21492.

Cohen, Paul A., et al., 2019. Cervical cancer. Lancet 393 (10167), 169–182. https://doi.org/10.1016/S0140-6736(18)32470-X.

Forbes, Neil S., 2010. Engineering the perfect (bacterial) cancer therapy. Nat. Rev. Cancer 10 (11), 785–794. https://doi.org/10.1038/nrc2934.

Ghaleh, Matineh Rahmani, et al., 2019. Evaluating the effects of Staphylococcus aureus enterotoxin B on the expression of BAX, p53, Caspase3, and Bcl-2 genes in gastric cancer cell line. Journal of BioScience & Biotechnology 8.2, 3719-1-10-20200513.

Hashemzehi, Rassoul, et al., 2018. Cloning and expression of nlpA gene as DNA vaccine candidate against Acinetobacter baumannii. Mol. Biol. Rep. 45 (4), 395–401. https://doi.org/10.1007/s11033-018-4167-y.

Hsu, Brenda, Marin, Maria C., Mcdonnell, Timothy J., 1995. Cell death regulation during multistep lymphomagenesis. Cancer Lett. 94 (1), 17–23. https://doi.org/10.1016/0304-3835(95)03836-l.

Iacovache, Ioan, Bischofberger, Mirko, Gisou van der Goot, F., 2010. Structure and assembly of pore-forming proteins. Curr. Opin. Struct. Biol. 20 (2), 241–246. https://doi.org/10.1016/j.sbi.2010.01.013.

Kale, Justin, Osterlund, Elizabeth J., Andrews, David W., 2018. BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ. 25 (1), 65–80. https://doi.org/10.1038/cdd.2017.186.

Karim, Sajjad, 2014. Clinicopathological and p53 gene alteration comparison between young and older patients with gastric cancer. Asian Pac. J. Cancer Prev. 15 (3), 1375–1379. https://doi.org/10.7314/apjcp.2014.15.3.1375.

Leschner, Sara, Weiss, Siegfried, 2010. Salmonella—allies in the fight against cancer. J. Mol. Med. 88 (8), 763–773. https://doi.org/10.1007/s00109-010-0636-z.

Minton, Nigel P., 2003. Clostridia in cancer therapy. Nat. Rev. Microbiol. 1 (3), 237–242. https://doi.org/10.1038/nrmicro777.

Mo, Yiqun, Wan, Rong, Zhang, Qunwei, 2012. Application of reverse transcription-PCR and real-time PCR in nanotoxicity research. In: Nanotoxicity. Humana Press, Totowa, NJ, pp. 99–112. https://doi.org/10.1007/978-1-62703-002-1_7.

Olatavi, Z.N., Milliman, C.L., Korsmeyer, S.J., 1994. Bcl-2 heterodimerizes in vivo with a conserved homologue BAX that accelerates cell death. Cell 74, 609–619. https://doi.org/10.1016/0092-8674(93)90509-o.

Pahle, Jessica, Walther, Wolfgang, 2016. Bacterial toxins for oncoleaking suicidal cancer gene therapy. Current Strategies in Cancer Gene Therapy 95–110. https://doi.org/10.1007/978-3-319-42934-2_7.

Pahle, Jessica, et al., 2017. Rapid eradication of colon carcinoma by Clostridium perfringens enterotoxin suicidal gene therapy. BMC Cancer 17 (1), 1–14. https://doi.org/10.1186/s12885-017-3123-x.

Park, Kyoungsook, et al., 2012. Hypoxia inhibition of camptothecin-induced apoptosis by Bax loss. Biologia 67 (3), 616–621. https://doi.org/10.2478/s11756-012-0037-6.

Pearson, Jaclyn S., Murphy, James M., 2017. Down the rabbit hole: is necroptosis truly an innate response to infection? Cell. Microbiol. 19 (8), e12750 https://doi.org/10.1111/cmi.12750.

Pietrantonio, Filippo, et al., 2013. A review on biomarkers for prediction of treatment outcome in gastric cancer. Anticancer Res. 33 (4), 1257–1266, 2434/426126/1546213/1257.

Ramachandran, Girish, 2014. Gram-positive and gram-negative bacterial toxins in sepsis: a brief review. Virulence 5 (1), 213–218. https://doi.org/10.4161/viru.27024.

Roberts, A.I., et al., 2000. Staphylococcal enterotoxin B induces potent cytotoxic activity by intraepithelial lymphocytes. Immunology 101 (2), 185–190. https://doi.org/10.1046/j.1365-2567.2000.00088.x.

Sabzehali, Fattaneh, Azimi, Hadi, Goudarzi, Mehdi, 2017. Bacteria as a vehicle in cancer therapy and drug delivery. Archives of Advances in Biosciences 8 (1), 52–59. https://doi.org/10.22037/jps.v8i1.13574.

Schiffman, Mark, Solomon, Diane, 2013. Cervical-cancer screening with human papillomavirus and cytologic cotesting. N. Engl. J. Med. 369 (24), 2324–2331. https://doi.org/10.1056/NEJMcp1210379.

Smith, James L., Bayles, Darrell O., 2006. The contribution of cytolethal distending toxin to bacterial pathogenesis. Crit. Rev. Microbiol. 32 (4), 227–248. https://doi.org/10.1080/10408410601023557.

Song, Shiyu, Vuai, Miza S., Zhong, Mintao, 2018. The role of bacteria in cancer therapy–enemies in the past, but allies at present. Infectious Agents and Cancer 13 (1), 1–7. https://doi.org/10.1186/s13027-018-0180-y.

Srivastava, Saumya S., et al., 2009. Membrane bound monomer of staphylococcal α-hemolysin induces caspase activation and apoptotic cell death despite initiation of membrane repair pathway. PLoS One 4 (7), e6293. https://doi.org/10.1371/journal.pone.0006293.

Torchinsky, Miriam Beer, Garaude, Johan, Blander, J. Magarian, 2010. Infection and apoptosis as a combined inflammatory trigger. Curr. Opin. Immunol. 22 (1), 55–62. https://doi.org/10.1016/j.coi.2010.01.003.

Toshiyuki, Miyashita, Reed, John C., 1995. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 80 (2), 293–299. https://doi.org/10.1016/0092-8674(95)90412-3.

Tsikouras, Panagiotis, et al., 2016. Cervical cancer: screening, diagnosis and staging. J buon 21 (2), 320–325. pubmed.ncbi.nlm.nih.gov.

Tsujitani, Shunichi, et al., 2012. Relationship between expression of apoptosis-related proteins and the efficacy of postoperative chemotherapy in patients with T3 gastric cancer. Surg. Today 42 (3), 225–232. https://doi.org/10.1007/s00595-011-0062-z.

Ye, Yan-Wei, et al., 2012. The correlations between the expression of FGFR4 protein and clinicopathological parameters as well as prognosis of gastric cancer patients. J. Surg. Oncol. 106 (7), 872–879. https://doi.org/10.1002/jso.23153.

Zacharski, L.R., Sukhatme, V.P., 2005. Coley's toxin revisited: immunotherapy or plasminogen activator therapy of cancer? J. Thromb. Haemost. 3 (3), 424–427. https://doi.org/10.1111/j.1538-7836.2005.01110.x.

Zhao, Ming, et al., 2012. Efficacy against lung metastasis with a tumor-targeting mutant of Salmonella typhimurium in immunocompetent mice. Cell Cycle 11 (1), 187–193. https://doi.org/10.4161/cc.11.1.18667.