Texosome-anchored superantigen triggers apoptosis in original ovarian cancer cells

Article *in* Medical Oncology · January 2015

DOI: 10.1007/s12032-014-0409-6 · Source: PubMed

Med Oncol (2015) 32:409
DOI 10.1007/s12032-014-0409-6

ORIGINAL PAPER

Texosome-anchored superantigen triggers apoptosis in original ovarian cancer cells

Hamideh Mahmoodzadeh Hosseini · Jafar Soleimanirad · Elnaz Mehdizadeh Aghdam · Mohsen Amin · Abbas Ali Imani Fooladi

Received: 21 November 2014 / Accepted: 24 November 2014

Abstract Texosomes, nano-endosomal vesicles, are candidates for cancer immunotherapy due to their immunostimulating properties. We designed a new structure based on texosome and staphylococcal enterotoxin B (SEB) and assessed its cytotoxic impact on an ovarian cell line. Texosomes were isolated from tumor cells, and SEB was anchored onto by protein transfer method. MTT assay and Hoechst staining were used to identify the cytotoxic and apoptotic effects of this compound on treated cells with different concentrations of texosome–SEB (TEX–SEB). Moreover, the expression rate of *bcl-2, bax, bak, bcl-xl* and the activity of caspase-3 and caspase-9 were investigated. Treatments of the cells with 0.5, 2.5 and 10 μg/100 μl TEX–SEB were significantly cytotoxic within 24 h ($p < 0.001$). Hoechst staining revealed that all tested concentrations caused apoptosis after 24 h compared with the control cells ($p < 0.001$). Furthermore, it was found that treatment with all examined concentrations of TEX–SEB enhanced caspase-9 activity after 24 and 48 h, while caspase-3 activity was increased upon treatment with only 0.5 and 2.5 μg/100 μl of TEX–SEB after 24 h ($p < 0.001$). None of the concentrations of TEX–SEB affected the expression of the cancer-promoting genes. Our construct, the TEX–SEB, is a new model being able to create cytostatic properties on cancer cells.

Keywords Ovarian cancer · Apoptosis · Texosome · *Staphylococcus* enterotoxin B · Immunotherapy

Introduction

Ovarian cancer is the third most common type of gynecological cancer and the leading cause of mortality of the patients. There are still many obscurities regarding ovarian cancer such as mechanisms and signaling pathways in the development, progression, invasion and metastasis of the tumor tissue [1]. Currently, several therapeutic strategies are applied. Yet, they are not efficient enough due to the poor tumor diagnosis at the advanced stages. Cytoreductive surgery and platinum-based chemotherapy are two common therapies for treating progressive epithelial ovarian cancer. Despite initial improvement, the rate of recurrence and progression is considerably high because of chemoresistance [2, 3]. This enforces the search for designing and innovating new strategies to overcome the ovarian cancer.

One attractive topic in tumor immunotherapy is the use of exosomes as cell-free and specific tools for combating cancer [4]. Exosomes are membranous endosomal vesicles with the size of 30–100 nm, which are secreted from various types of normal and cancer cells [5]. These vesicles reversely bud from multivesicular bodies, fuse with the plasma membrane and finally are released into the extracellular medium [5, 6]. Tumor-derived exosomes are known as texosomes. Texosomes carry various cytosolic and membranous tumor antigens accompanied with

H. Mahmoodzadeh Hosseini · A. A. Imani Fooladi (✉)
Applied Microbiology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
e-mail: imanifouladi.a@gmail.com; imanifouladi.a@bmsu.ac.ir

J. Soleimanirad
Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

E. Mehdizadeh Aghdam
Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

M. Amin
Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Published online: 02 December 2014

molecules participated in antigen presentation, making them a promising candidate for cancer therapy. They are capable of priming T cells by offering antigens to antigen-presenting cells such as dendritic cells [7, 8]. On the other hand, texosomes consist of large amounts of antigens. This increases the possibility of anergy which can be resolved by using adjuvants. Besides, local administration of the texosomes fused to cytostatic agents proposes an efficient therapeutic approach for established tumors.

There are some cancer studies on the preventive and therapeutic effects of texosomes [8]. A few studies evaluated texosome effect on the fate of tumor cells. Two recent studies used the texosomes isolated from the culture medium of pancreatic cancer cells. They showed that the texosomes could activate mitochondrial-dependent apoptosis and enhanced caspase-3 and caspase-9 activities. However, it was reported that texosomes from MIA Paca-2 cells, poorly differentiated pancreatic cancer cell line, had no effect on cell proliferation and cell death [9, 10]. Additionally, our recent works revealed that SEB-anchored texosome was cytotoxic for pancreatic [11] and breast cancer cell lines [12].

Current tumor cell death inducers are chemotherapeutic compounds, which usually suppress the immune system. Superantigens as well as T cell activators could be effective substances for this purpose. SEB as a potent superantigen can stimulate T cell proliferation and activation through attaching to major histocompatibility class II (MHC II) molecules on the surface of antigen-presenting cells. The SEB–MHC II complex occurs outside of the antigen-binding site and attaches to the variable region of β-chain of T cell receptor [13, 14]. Several previous studies have found that SEB has the potential to induce antitumor immune responses [15–17]. In addition, the SEB impact on the extrinsic apoptosis pathway, also named Fas-mediated apoptotic pathway, and modifies the expression of the proteins involved in this pathway [18].

In this study, we designed a structure based on texosome carrying the tumor antigens and anchored them to SEB as a potent superantigen. Based on our hypothesis, the effect of the conjugate would be twofold: the cytostatic effect on original tumor ovarian cancer cells and the immunostimulating effects.

Materials and methods

Cell culture

A SKOV-3 cell line, an epithelial-like ovary adenocarcinoma cell line, was purchased from the Pasteur Institute (Tehran, Iran). Cells were grown in RPMI 1640 medium supplemented with 10 % fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in a 5 % CO_2 atmosphere. All the cell culture reagents were obtained from PAA company (the Netherlands).

Exosome purification

SKOV-3 cells were grown in T175 flasks (Nest, the Netherlands). After reaching 85–90 % confluence, the attached cells were washed three times with phosphate-buffered saline (PBS) and then the fresh medium without FBS was added to each flask. After 48 h, the supernatant of each flask was transferred to a fresh tube and TEXs were purified according to the method described by Battke et al. [19]. Firstly, the supernatant was sequentially centrifuged at 2,000×g for 10 min and 10,000×g for 30 min to remove dead cells and debris. Then, it was filtered using a 0.22-µm filter (GSV Filter Technology, USA). The filtrate was concentrated using a Centricon Plus-70 centrifugal filter device (Millipore, USA). Finally, the ultracentrifugation was carried out in a TL-100 rotor at 100,000×g for 1 h to pellet down the ultra filtrated concentrate. The obtained pellet was re-suspended in 1 ml PBS and stored at −80 °C for the following examinations. The protein concentration of purified TEXs was measured at 280 nm using a Nanodrop spectrophotometer (Thermos, USA).

Transmission electron microscopy

To evaluate the morphology and size of purified TEXs, transmission electron microscopy (TEM) was used. Five micrograms of TEX solution in PBS was treated with an equal amount of 2.5 % glutaraldehyde and was transferred onto a formware/carbon coated grid (IBB, Iran). Following incubation for 20 min at room temperature, the grid was transferred to 50 µl of uranyl oxalate pH 7 (Merck, Germany) for 5 min and washed with PBS. Then, methyl cellulose/uranyl acetate (Merck, Germany) was added to the grid and allowed to stand on ice for 10 min. The excess fluid was removed by Whatman filter paper No. 1. The grid was air-dried, and the morphology and size of TEXs were observed by TEM (LEO906, Germany) at 80 kV.

SDS-PAGE and Western blotting

Hsp-70 (one of the most common markers of TEXs) was detected using Western blotting (21). An equal amount of SKOV-3 cell lysate (lysis buffer containing 10 mM triton X-100 pH 7.5 and 150 mM NaCl) and purified TEXs were separated by SDS-PAGE containing 12.5 % polyacrylamide (Merck, Germany) plus 0.1 % SDS (Merck, Germany). Then, proteins were electrophoretically transferred onto a PVDF (Bio-Rad, USA) and then blocked using 5 % skimmed milk solution in Tris-buffered saline with 0.1 %

Tween-20 (TTBS) and incubated overnight at 4 °C. To specifically detect Hsp-70, the membrane was incubated with mouse anti-Hsp-70 primary antibody (Abcam, USA) for 2 h at room temperature and then washed by TTBS three times. In the next step, HRP-conjugated anti-mouse IgG (Razi, Iran) was added to the membrane and incubated for 1 h at room temperature. After washing, to visualize the protein of interest, a chromogenic stain, DAB/$NiCl_2$, was utilized.

Protein anchorage of SEB on exosomes

To anchor SEB on TEXs, the protocol described by McHugh et al. [20] was utilized. Briefly, 10 μg SEB (Sigma-Aldrich, Germany) was added to 100 μg of purified TEXs in 100 μl PBS. The mixture was placed on a shaker at 1,000 rpm for 4 h at 37 °C and was filtered using Ultrafree-0.5 Biomax 100k (Millipore, USA) at 3,000×*g* for 20 min to remove the unbound SEB. The SEB-anchored TEXs were named TEX–SEB.

Proliferation assay

The effect of TEX–SEB on the proliferation of SKOV-3 cells was investigated by MTT assay after 24 and 48 h. Briefly, 10^4 cells were seeded on each well of a 96-well plate and incubated as explained in the cell culture subheading. After 24 h, the cells were treated with four different concentrations of TEX–SEB including 0.5, 2.5, 5 and 10 μg/100 μl in culture medium. An equal amount of TEXs (without modification), SEB and the mixture of SEB + TEXs were used as controls and named TEX, SEB and TEX + SEB, respectively. In addition, the cells treated with PBS were considered as negative control. Twenty microliters MTT reagent (5 mg/ml) (Sigma-Aldrich, Germany) was added to each well and incubated for 4 h at 37 °C. The supernatants were replaced with 100 μl dimethyl sulfoxide (Sigma-Aldrich, Germany). Then, the optical density of each well was measured using a microplate reader (Tecan, Switzerland) at 570 nm. All the tests were carried out in triplicate.

Apoptosis assay

The induction of apoptosis by TEX–SEB was detected by Hoechst 33258 staining method [17]. This stain specifically binds to the A–T regions of intact DNA strands and forms a fluorescent complex. The fragmented nuclear DNA does not create fluorescent signals. As described above, cells were treated with four concentrations, including 0.5, 2.5, 5 and 10 μg/100 μl of TEX–SEB. TEX, SEB, TEX + SEB and PBS were tested as controls. After 24 h, cells of each well were detached by trypsin/EDTA and fixed on a slide by 4 % formaldehyde solution. Cells were stained with 1 % Hoechst 33258 and observed by fluorescent microscope (Micros, Austria) under the high-power field (400×) using UV beam. Thousand cells were randomly selected, and the number of apoptotic cells per 1,000 cells was determined. Finally, the apoptotic index of each well was calculated according to the following formula:

$$\text{Apoptotic index} = \frac{\text{The number of apoptotic cells} \times 100}{\text{Total number of counted cells}}$$

All tests were performed in triplicate.

Assessment of caspase-3 and caspase-9 activity

Caspase activity following TEX–SEB treatment was evaluated via caspase-3 and caspase-9 colorimetric assay kits (Genscript, USA) according to the manufacturers' instruction. Briefly, 3×10^6 treated cells were lyzed by 50 μl cold lysis buffer. After 60 min, each sample was centrifuged at 10,000 rpm for 1 min at 4 °C. A clear supernatant was transferred to a clean tube on ice, and the protein concentration was measured by Nanodrop spectrophotometer at 280 nm. Fifty microliters of 2× reaction buffer was added to an equal volume of cell lysate containing 100–200 μg protein followed by the addition of 5 μl caspase-3 substrate (DEVD-*p*NA). After 4 h incubation at 37 °C, the extinction value of each test was measured at 400 nm using a microplate reader (Techan, Switzerland). The relative changes of caspase-3 activity were determined by calculating the optical density $(OD)_{test}$/$(OD)_{negative\ control}$. The relative activity of caspase-9 was measured in a similar way. All tests were performed in triplicate.

Gene expression analysis

To assess the effect of the TEX–SEB on the expression of some genes involved in apoptosis, the reverse transcriptase-polymerase chain reaction (RT-PCR) was used. After exposing the cells to 0.5, 2.5, 5 and 10 μg/100 μl of TEX–SEB for 48 h, the total mRNA was isolated using the RNX-Plus kit (Cinnagen, Iran) according to the manufacturer's instruction. The mRNA was precipitated in isopropanol solution and finally resususpended in 20 μl DEPC water. The concentration of mRNA was measured by the Nanodrop spectrophotometer (Thermos, USA). Bioneer kit (Takara, Japan) was used to synthesize related cDNA. In each RT reaction, 1 μg of isolated mRNA was converted to cDNA using the M-MLV reverse transcriptase, random hexamers and oligo dT. In the second step, the expression of the *bak, bax, bcl*$_2$*, bcl-x*$_l$ genes were determined by PCR using specific primer sets for each gene. The expression of

the *β-actin* was assessed as an internal control. The sequences, annealing temperature and the product sizes of each primer are listed in Table 1. One microliter of each cDNA was amplified in 20 µl of mixture reaction containing 10× reaction buffer, 0.2 mM of the deoxynucleoside triphosphates (dNTPs), 2.5 mM $MgCl_2$, 10 ρmol of each primers and 1.5 U of Taq DNA Polymerase (Cinnagen, Iran). PCR procedure was performed by thermocycler (Eppendorf, Germany) with an initial denaturation step of 6 min at 94 °C, 30 cycles of 30 s at 94 °C, 45 s at specified annealing temperature for each primer and 45 s at 72 °C followed by 5 min final extension at 72 °C. The PCR products were visualized using electrophoresis on 1.5 % agarose gel and staining with ethidium bromide. The density of product bands was measured by the ImageJ software (National Institutes of Health, USA).

Statistical analysis

Data obtained from all tests were assessed by the non-parametric Mann–Whitney *U* test using SPSS.15 software (SPSS, Chicago, IL, USA). p value < 0.05 was considered statistically significant.

Results

Identification of texosomes isolated from SKOV-3 cells

Texosomes was used to evaluate the size and morphology of TEXs. As represented in Fig. 1, the TEXs were round membranous vesicles with 40–150 nm diameter. The presence of Hsp-70, an exosomal protein marker, was assessed by Western blotting. We observed that purified TEXs and SKOV-3 cell lysates contained the Hsp-70 protein. Figure 2 displays a view of the PVDF membrane containing the protein marker.

Cell proliferation analysis (MTT assay)

SKOV-3 cells were exposed to different concentrations of the TEX–SEB for two periods, 24 and 48 h, and cell proliferation was examined using MTT assay. After 24 h, 0.5, 2.5 and 10 µg/100 µl of TEX–SEB significantly reduced the proliferation of the cells in comparison with negative controls (67.49, 73.56 and 85.35 % reduction, respectively) ($p < 0.001$). The most efficient concentration was 0.5 µg/100 µl of TEX–SEB (Fig. 3a). Moreover, 0.5 µg/100 µl of TEX–SEB significantly decreased the cell proliferation compared to the treatment with equal concentration of SEB or the mixture of TEX and SEB ($p < 0.001$). Furthermore, 10 µg/100 µl TEX–SEB significantly attenuated cell proliferation in comparison with the treatment with equal concentration of TEX. Although 48-h

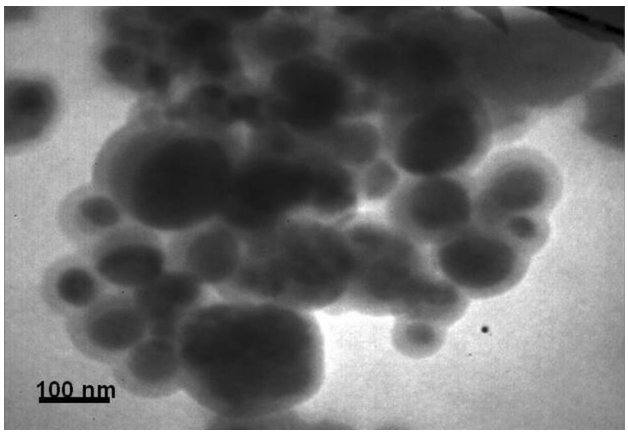

Fig. 1 Transmission electron microscopy illustration of the isolated texosomes. Negative-stained purified texosomes were observed by TEM (×60,000). The texosomes' diameter is 40–150 nm

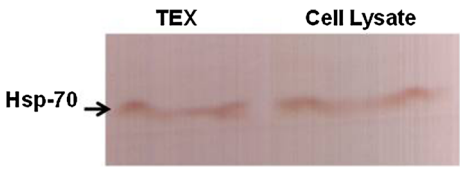

Fig. 2 Western blotting of texosomal protein markers. An equal amount of lysate of Skov-3 cells and their texosomes were separated by SDS-PAGE, and the presence of Hsp-70 protein was identified using a specific antibody, anti-Hsp-70. As shown in this figure, both TEX (an indicator for exosome) and the cell lysate contain Hsp-70

Table 1 Sequences of primers used for the related PCR reactions

Gene	Sense primer (5′–3′)	Anti-sense primer (5′–3′)	Annealing temperature (°C)	Product size (bp)	Reference
bcl-2	CGACTTCGCCGAGATGTCCAGCCAG	ACTTGTGGCCCAGATAGGCACCCAG	56	388	22
bcl-xl	GGAGCTGGTGGTTGACTTTCT	CCGGAAGAGTTCATTCACTAC	54	379	23
bax	AGGGTTTCATCCAGGATCGAGCAG	ATCTTCTTCCAGATGGTGAGCGAG	51	490	24
bak	TCAACCGACGCTATGACT	TCTTCGTACCACAAACTGG	52	368	25
β-Actin	TCATGAAGATCCTCACCGAG	TTGCCAATGGTGATGACCTG3	58	190	26

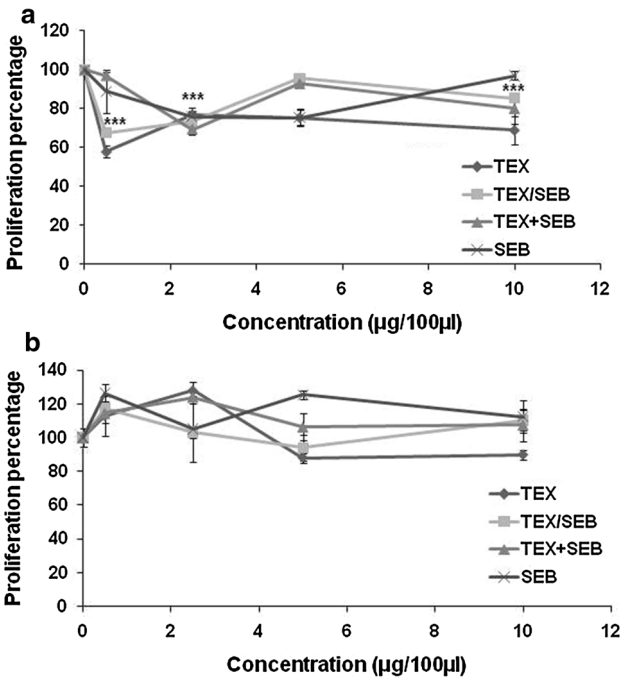

Fig. 3 The cell proliferation rate of Skov-3 treated with the TEX–SEB after 24 (**a**) and 48 h (**b**). The cells were treated with 0.5, 2.5, 5 and 10 µg/100 µl of TEX–SEB for 24 and 48 h. Also, equal amount of TEX, TEX + SEB, SEB and PBS were examined as controls. After 24 h, significantly reduction in proliferation rate was determined at concentrations of 0.5 and 2.5 µg/100 µl of TEX–SEB ($p < 0.001$). After 48 h, the TEX–SEB treatment had no effect on cell proliferation

treatment with TEX–SEB led to a negative effect on cell proliferation, it was much less than the proliferation reduction within 24-h incubation. Moreover, no statistically significant difference in cell proliferation was found compared to the control group (Fig. 3b).

Apoptosis assay

To survey the apoptotic index of TEX–SEB, the treated cells were stained with Hoechst 33258. Figure 4 displays a view of the apoptotic and non-apoptotic cells using fluorescent microscope. Findings from Hoechst staining test indicated the TEX–SEB caused apoptosis at all the concentration examined in this study ($p < 0.001$). As illustrated in Fig. 5, the rate of apoptosis caused by 0.5, 2.5, 5 and 10 µg/100 µl of TEX–SEB was 27.1, 26.26, 25.2 and 25.93 % compared to negative control, respectively. 0.5 and 2.5 µg/100 µl of TEX–SEB had the most effective apoptotic index. Furthermore, treating the cells with all tested TEX–SEB concentrations remarkably led to apoptosis compared to the treatment with equal concentration of TEX + SEB and SEB ($p < 0.001$). Moreover, 5 and 10 µg/100 µl of TEX–SEB significantly raised the apoptosis rate compared to the treatment with equal

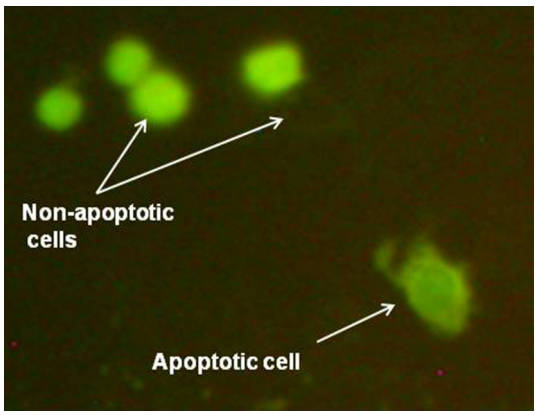

Fig. 4 A Hoechst staining illustration. Stained Skov-3 observed under fluorescent microscope (×400). Cells with the degraded and fragmented chromosomes are apoptotic and demonstrate a colorless and non bright nuclei, whereas intact and non-apoptotic cells have dense chromosomes and show completely stained and bright nuclei

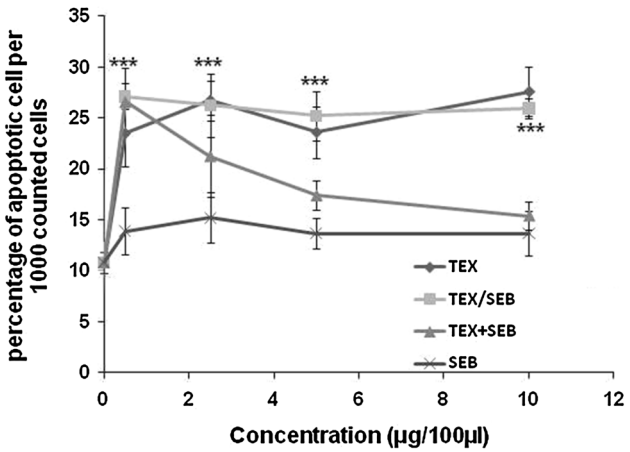

Fig. 5 Analysis of apoptosis in Skov-3 after treatment with four different concentrations (0.5, 2.5, 5 and 10 µg/100 µl) of TEX–SEB for 24 h. Also 0.5, 2.5, 5 and 10 µg/100 µl of TEX, TEX + SEB, SEB and PBS were examined as controls. As seen in this Figure, all concentrations can significantly induce apoptosis in the Skov-3 cells after 24 h ($p < 0.001$)

concentrations of the mixture of TEX and SEB ($p < 0.001$, $p < 0.05$).

Caspase activity assay

Specific colorimetric kits were used to investigate whether TEX–SEB could augment the activity of caspase-3 and -9. After 24-h exposure to TEX–SEB, significant increase was found in the activity of caspase-9 at all tested concentrations (Fig. 6a), while treatment with 0.5 and 2.5 µg/100 µl of TEX–SEB increased caspase-3 activity (Fig. 7a, $p < 0.001$). Unlike caspase-3 (Fig. 7b), changes in the activity of caspase-9 was viewed at all concentrations after 48-h exposure to TEX–SEB (Fig. 6b, $p < 0.001$).

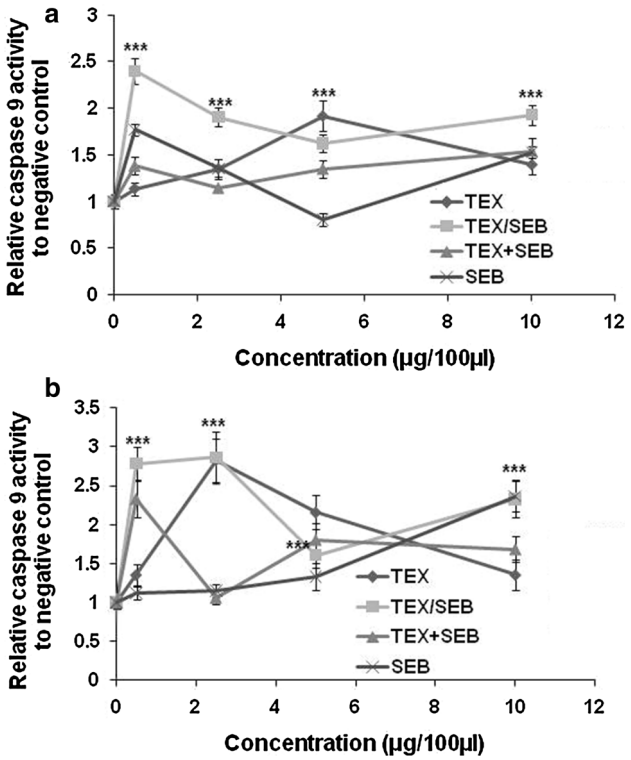

Fig. 6 Effect of TEX–SEB on the induction of caspase-9 activity in Skov-3 cells. Caspase activity was quantified by the colorimetric method in the Skov-3 cells following treatment with the four different concentrations of TEX–SEB (0.5, 2.5, 5 and 10 µg/100 µl) after 24 and 48 h. TEX–SEB increased the activity of caspase-9 at all concentration after 24 (**a**) and 48 h (**b**) ($p < 0.001$)

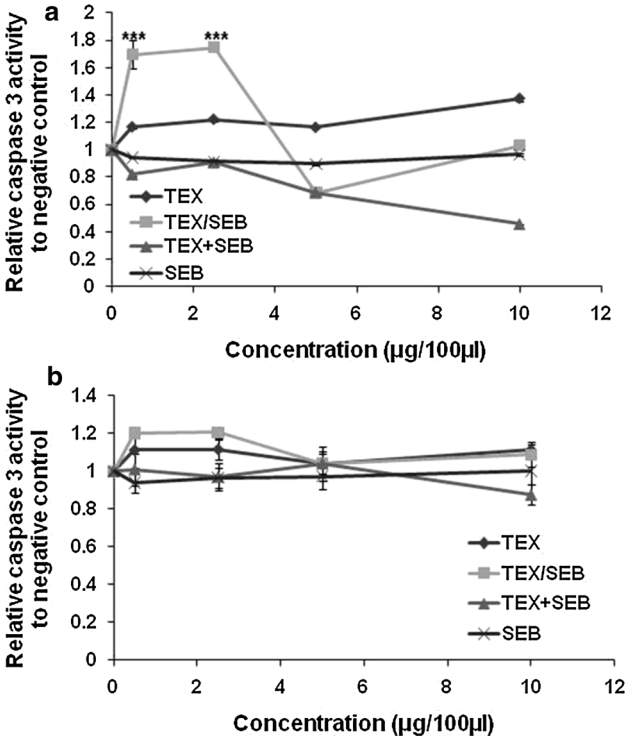

Fig. 7 Effect of EXO–SEB on the induction of caspase-3 activity in Skov-3 cells. Caspase activity was quantified by the colorimetric method in the Skov-3 cells following treatment with the four different concentrations of EXO–SEB (0.5, 2.5, 5 and 10 µg/100 µl) after 24 and 48 h. TEX–SEB increased the activity of caspase-3 after 24 h (**a**) at the concentration of 0.5, 2.5 and 5 µg/100 µl ($p < 0.001$) but had no effect after 48 h (**b**)

Gene expression

The effect of TEX–SEB treatment on the expression of anti-apoptotic genes (*bcl-2* and *bcl-xl*) as well as pro-apoptotic genes (*bax* and *bak*) was determined by semi-quantitative RT-PCR. Our data indicated no significant change in the expression of *bax*, *bak*, *bcl-2* and *bcl-xl* mRNA levels (Fig. 8).

Discussion

Exosome display uses are a novel approach to manipulate the molecular composition and to confer new biological functions to the structure. Recently, this technique has been used to design specific antibodies against tumoral epitopes and biomarkers [21]. Moreover, this strategy provides an opportunity to design new, efficient diagnostic and therapeutic tools [6]. Here, this technique was used to synthesize a two-component structure containing texosomes (derived from tumoral cells) and SEB for inducing cytostatic effect on the target cells. We conjugated the components using the anchoring method that depends on the membrane characteristic of texosomes. The presence of high levels of lipid compounds, including sphingomyelin, cholesterol and glycolipid along with tetraspanin proteins facilitates the anchorage event through protein transfer protocol [22, 23]. This method has several advantages such as reducing systemic toxicity upon exposure to SEB, using lower concentrations of SEB to stimulate appropriate immune response [24], shortening the binding time of the two components compared to the expression of the relevant fusion proteins via cloning. Also, this method is not affected with environmental factors such as temperature and incubation time during anchoring process [25]. In addition, two components of anchored structures simultaneously influence on a single site. Since texosomes are stable vesicles, the anchorage of the staphylococcal toxin makes it more stable compared to the free toxin. Therefore, it is asserted that the anchorage method is an appropriate protocol for expressing foreign proteins on texosomes [24].

In this study, we designed a conjugate based on exosome structure that is able to activate cytostatic signals in target cells along with the induction of particular antitumor immune responses. The TEX–SEB is made up of two parts: tumor cell-derived exosomes and anchored SEB as a

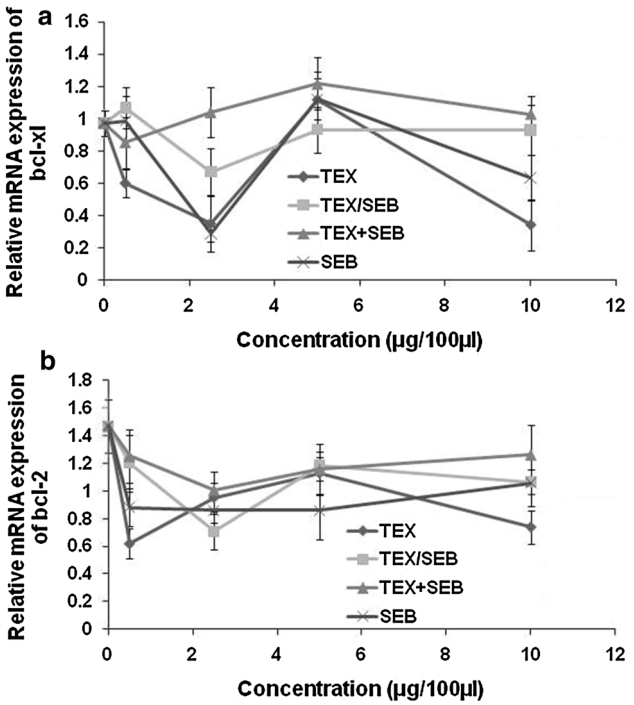

Fig. 8 Effect of TEX–SEB on the expression of *bcl-xl,* and *bcl-2* in Skov-3. The mRNA levels of the mentioned genes were relatively quantified by RT-PCR in the Skov-3 cell treated with the four different concentrations of TEX–SEB (0.5, 2.5, 5 and 10 µg/100 µl) after 48 h. *β-actin* was examined as a housekeeping gene, and consequently, the results were shown as a ratio of desired gene expression/*β-actin* expression. EXO–SEB showed no effect on the expression of *bcl-xl* (**a**) and *bcl-2* (**b**)

superantigen. The combination of SEB and exosomes were exerted via the glycosylphosphatidylinositol anchorage using protein transfer method [20]. The presence of high levels of lipid raft domain, rich in sphingomyelin and cholesterol, on the surface of exosomes [10] provides functional regions to contribute to the enhancement of apoptotic signals. Furthermore, SEB can persuade apoptosis due to the FAS/FASL system [18]. Our results clearly suggested the cytostatic properties of TEX–SEB in SKOV-3 cells. Data from MTT assay revealed the cytotoxic impact of TEX–SEB on the SKOV-3 cells. Moreover, findings from Hoechst staining showed that the apoptotic effects upon exposure to TEX–SEB are dose dependent. This finding corresponds with the results of our previous works showing the apoptotic activity of SEB-anchored exosome derived from breast cancer [12] and pancreatic cancer cell lines [11]. In those studies, we observed the activation of mitochondrial-dependent apoptosis pathway owing to increasing expression of pro-apoptotic genes involved in *bcl2* family and caspase-3 activity. Additional data reported by Ristorcelli and colleagues [10] showed anti-proliferative effect of tumor-derived exosome on some pancreatic cancer cell lines because of reducing the level of *hes-1* and stimulating the PTEN and GSK-3β activation [9]. Peng et al. [1] findings were inconsistent with our data. They pointed out that exosomes derived from ascite samples of patients suffered from ovarian cancer had no significant impact on the apoptosis and cell death together with the proliferation of ovarian cancer cells after exposure to different volumes of exosomes. Furthermore, they suggested that the ascite exosome probably includes neither growth nor death stimulator substances for tumor cells. But they declared ascite exosomes can activate apoptosis in treated peripheral polymorphonuclear cells owing to the presence of FASL and TRAIL protein within this exosomes. In contrast to Peng et al. [1], we observed the anti-proliferative and apoptotic behavior of TEX–SEB on SKOV-3 cells.

In this study, we showed a significant rise in the activity of caspase-3 and -9. Ristrocelli et al. [10] suggested exosome-enriched cholesterol, ceramide and sphingomyelin cause caspase-3 and caspase-9 activation. In addition, our previous work indicated the increased activity of described caspases after exposure to exosomel/staphylococcal enterotoxin B [12]. But here we showed no changes in the expression level of pro-apoptotic and anti-apoptotic genes tested here. Previous report intimated that texosome and its derivatives could induce the mitochondrial-dependent apoptotic pathway [10, 12]. High level of bax leads to inactivation of the protective function of *bcl-2*. Further, bax stimulates releasing of cytochrome *c* from mitochondria and finally results in apoptosis [26]. The outcomes of caspase activity analysis confirmed our findings from Hoechst staining. Various death signals are commonly able to activate caspase-3 that it consequently leads the cleavage of numerous significant proteins, which corresponds with DNA fragmentation and cellular apoptotic changes [27]. It should be noted that except mitochondria, cytoplasmic organelles such as reticulum endoplasm is able to trigger and activate apoptosis pathways. In these pathways, the organelle leads to generate oxidative stress, which can influence outer membrane of mitochondria, release the cytochrome *c* and consequently the conversion of procaspase-9 to activate caspase-9 [28, 29]. Since our results revealed no alteration in the expression of *bcl-2* family, the activation of caspase-9 may be due to other signaling events exemplified above. Therefore, further studies require to investigate other apoptotic signaling pathways after exposure to TEX–SEB.

Conclusion

In conclusion, exosome display can be an appropriate method to create a two-component model for apoptotic therapy and provoking apoptosis. The presence of exosome

and its lipid rafts in this structure provides the possibility of binding to tumor cells. Besides, the discrepant behavior of different cells to exosomes derive from the same cells suggested that each cell line secretes the exosome with the unique properties containing special protein and lipid content. Obviously, further studies on the molecular and cellular mechanisms are required to support our results.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Peng P, Yan Y, Keng S. Exosomes in the ascites of ovarian cancer patients: origin and effects on anti-tumor immunity. Oncol Rep. 2011;25(3):749–62. doi:10.3892/or.2010.1119.
2. Alberts DS. Treatment of refractory and recurrent ovarian cancer. Semin Oncol. 1999;26(1 Suppl 1):8–14.
3. McGuire WP, Ozols RF. Chemotherapy of advanced ovarian cancer. Semin Oncol. 1998;25(3):340–8.
4. Rieger J, Freichels H, Imberty A, Putaux JL, Delair T, Jérôme C, et al. Polyester nanoparticles presenting mannose residues: toward the development of new vaccine delivery systems combining biodegradability and targeting properties. Biomacromolecules. 2009;10(3):651–7. doi:10.1021/bm801492c.
5. Hosseini HM, Fooladi AA, Nourani MR, Ghanezadeh F. Role of Exosome in infectious disease. Inflamm Allergy Drug Targets. 2013;12(1):29–37.
6. Schorey JS, Bhatnagar S. Exosome function: from tumor immunology to pathogen biology. Traffic. 2008;9(6):871–81. doi:10.1111/j.1600-0854.2008.00734.x.
7. Cho JA, Yeo DJ, Son HY, Kim HW, Jung DS, Ko JK, et al. Exosomes: a new delivery system for tumor antigens in cancer immunotherapy. Int J Cancer. 2005;114(4):613–22. doi:10.1002/ijc.20757.
8. Tan A, De La Peña H, Seifalian AM. The application of exosomes as a nanoscale cancer vaccine. Int J Nanomed. 2010;5:889–900. doi:10.2147/ijn.s13402.
9. Ristorcelli E, Beraud E, Mathieu S, Lombardo D, Verine A. Essential role of Notch signaling in apoptosis of human pancreatic tumoral cells mediated by exosomal nanoparticles. Int J Cancer. 2009;125(5):1016–26. doi:10.1002/ijc.24375.
10. Ristorcelli E, Beraud E, Verrando P, Villard C, Lafitte D, Sbarra V, et al. Human tumor nanoparticles induce apoptosis of pancreatic cancer cells. FASEB J. 2008;22(9):3358–69. doi:10.1096/fj.07-102855.
11. Mahmoodzadeh Hosseini H, Ali Imani Fooladi A, Soleimanirad J, Reza Nourani M, Mahdavi M. Exosome/staphylococcal enterotoxin B, an anti tumor compound against pancreatic cancer. J BUON. 2014;19(2):440–8.
12. Mahmoodzadeh Hosseini H, Imani Fooladi AA, Soleimanirad J, Nourani MR, Davaran S, Mahdavi M. Staphylococcal entorotoxin B anchored exosome induces apoptosis in negative estrogen receptor breast cancer cells. Tumour Biol. 2014;35(4):3699–707. doi:10.1007/s13277-013-1489-1.
13. Choi YW, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J. Interaction of Staphylococcus aureus toxin "superantigens" with human T cells. Proc Natl Acad Sci. 1989;86(22):8941–5.
14. Kappler J, Kotzin B, Herron L, Gelfand EW, Bigler RD, Boylston A, et al. V beta-specific stimulation of human T cells by staphylococcal toxins. Science. 1989;244(4906):811–3.
15. Imani Fooladi AA, Sattari M, Hassan ZM, Mahdavi M, Azizi T, Horii A. In vivo induction of necrosis in mice fibrosarcoma via intravenous injection of type B staphylococcal enterotoxin. Biotechnol Lett. 2008;30(12):2053–9. doi:10.1007/s10529-008-9805-3.
16. Imani Fooladi AA, Sattari M, Nourani MR. Synergistic effects between Staphylococcal enterotoxin type B and monophosphoryl lipid A against mouse fibrosarcoma. J BUON. 2010;15(2):340–7.
17. Fooladi AAI, Sattari M, Nourani MR. Study of T-cell stimulation and cytokine release induced by Staphylococcal enterotoxin type B and monophosphoryl lipid A. Arch Med Sci. 2009;3:335–41.
18. Higgs BW, Dileo J, Chang WE, Smith HB, Peters OJ, Hammamieh R, et al. Modeling the effects of a Staphylococcal Enterotoxin B (SEB) on the apoptosis pathway. BMC Microbiol. 2006;6:48. doi:10.1186/1471-2180-6-48.
19. Battke C, Ruiss R, Welsch U, Wimberger P, Lang S, Jochum S, et al. Tumour exosomes inhibit binding of tumour-reactive antibodies to tumour cells and reduce ADCC. Cancer Immunol Immunother. 2011;60(5):639–48. doi:10.1007/s00262-011-0979-5.
20. McHugh RS, Nagarajan S, Wang YC, Sell KW, Selvaraj P. Protein transfer of glycosyl-phosphatidylinositol-B7-1 into tumor cell membranes: a novel approach to tumor immunotherapy. Cancer Res. 1999;59(10):2433–7.
21. Delcayre A, Estelles A, Sperinde J, Roulon T, Paz P, Aguilar B, et al. Exosome display technology: applications to the development of new diagnostics and therapeutics. Blood Cells Mol Dis. 2005;35(2):158–68. doi:10.1016/j.bcmd.2005.07.003.
22. Escola JM, Kleijmeer MJ, Stoorvogel W, Griffith JM, Yoshie O, Geuze HJ. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. J Biol Chem. 1998;273(32):20121–7.
23. Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Möbius W, Hoernschemeyer J, et al. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem. 2003;278(13):10963–72. doi:10.1074/jbc.M207550200.
24. Xiu F, Cai Z, Yang Y, Wang X, Wang J, Cao X. Surface anchorage of superantigen SEA promotes induction of specific antitumor immune response by tumor-derived exosomes. J Mol Med. 2007;85(5):511–21. doi:10.1007/s00109-006-0154-1.
25. Nagarajan S, Anderson M, Ahmed SN, Sell KW, Selvaraj P. Purification and optimization of functional reconstitution on the surface of leukemic cell lines of GPI-anchored Fc gamma receptor III. J Immunol Methods. 1995;184(2):241–51.
26. Fadeel B, Orrenius S. Apoptosis: a basic biological phenomenon with wide-ranging implications in human disease. J Intern Med. 2005;258(6):479–517. doi:10.1111/j.1365-2796.2005.01570.x.
27. Sharief M, Gani ZH. Garden cress lepidium sativum seeds as oral contraceptive plant in mice. Saudi Med J. 2004;25(7):965–6.
28. Ferrari D, Pinton P, Campanella M, Callegari MG, Pizzirani C, Rimessi A, et al. Functional and structural alterations in the endoplasmic reticulum and mitochondria during apoptosis triggered by C2-ceramide and CD95/APO-1/FAS receptor stimulation. Biochem Biophys Res Commun. 2010;391(1):575–81. doi:10.1016/j.bbrc.2009.11.101.
29. Wlodkowic D, Skommer J, McGuinness D, Hillier C, Darzynkiewicz Z. ER-Golgi network—a future target for anti-cancer therapy. Leuk Res. 2009;33(11):1440–7. doi:10.1016/j.leukres.2009.05.025.