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Growth-inhibitory effects of TGFαL3-SEB chimeric protein on colon cancer cell line

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ABSTRACT

Background: TGFαL3-SEB chimeric protein is a synthetic protein, which is produced by combining the third loop (L3) of transforming growth factor-α (TGF-α) with staphylococcal enterotoxin type B. To the best of our knowledge, anti-cancer activity of this chimeric protein against colon cancer that overexpresses epidermal growth factor receptor (EGFR) has not yet been studied. Thus, in the present study, the anti-tumor effects of TGFαL3-SEB chimeric protein on HT-29 colon cancer cells were evaluated.

Materials and methods: The TGFαL3-SEB chimeric protein was previously designed and cloned in *Escherichia coli* (*E. coli*) [1,2]. The level of expression and the purity of this novel protein were examined for further analysis. For this purpose, the cells were treated with different concentrations (25, 50 and 75 μg/ml) of TGFαL3-SEB and then the proliferation was detected using the MTT assay. The apoptosis-inducing potential of TGFαL3-SEB in HT-29 and HEK-293 cells was evaluated by flow cytometry using Annexin V/PI double staining method; in addition, *bax/bcl2* mRNA ratio, caspase-3 and caspase-9 activity were also assessed.

Results: In the present study, TGFαL3-SEB chimeric protein was produced in *E. coli*. After effective purification, its growth inhibitory effect was evaluated. Our results indicated that the incubation of HT-29 colon cancer cell with 25, 50 and 75 μg/ml of TGFαL3-SEB for 24 h leads to significant reduction of proliferation in a dose-dependent manner ($P < 0.05$). Further analysis indicated that exposure of EGFR expressing HT-29 cells to TGFαL3-SEB leads to significant increase of the caspase-3 and caspase-9 activity in a concentration-dependent manner ($P < 0.05$). *Bax/bcl-2* ratio also confirmed that TGFαL3-SEB has the pro-apoptotic effect. Flow cytometry analysis of TGFαL3-SEB treated cells showed that in addition to apoptotic cells, necrotic cells were also increased significantly at the concentration of 25, 50 and 75 μg/ml ($P < 0.05$).

Conclusion: In conclusion, our results demonstrated that TGFαL3-SEB chimeric protein induced cell death through both mechanisms of apoptosis and necrosis in HT-29 colon cancer cells. This paper has highlighted that TGFαL3-SEB has the potential to target EGFR expressing cancer cell.

1. Introduction

Epidermal growth factor receptor (EGFR) is one of the most important cell receptors, which plays a key role in a wide variety of cell growth processes [3,4]. This receptor also called HER (human EGF receptor) or c-erbB1, is a transmembrane protein with intrinsic tyrosine kinase activity. This multifunctional protein also plays a vital role in cell division and apoptosis, cell differentiation, cell migration and organogenesis [5]. A growing body of studies revealed that dysregulation of EGFR, as well as mutations in different domains of this receptor is strongly associated with human tumorigenesis [6]. EGFR has also been reported to be overexpressed in a wide variety of human malignancies including colorectal, breast and non–small cell lung cancers [7–9].

Despite decades of research, colon cancer still remains the third most common cancer and the third most common cause of death from

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cancer in developing countries [10]. Statistical studies revealed that colorectal cancer (CRC) causes one million deaths worldwide every year [11]. Epidemiological investigations have demonstrated that the sporadic development of colorectal cancer is tightly associated with dietary habits and genetic factors, as well [12]. Among genetic factors, a large body of investigations has reported that EGFR subfamily is strongly involved in CRC development. For instance, EGFR is more likely to be expressed in most of the CRC tumors [13]. The exact mechanisms by which EGFR develops tumorigenesis is partially unknown, however, it has been shown that EGFR is involved in both cell cycle dysregulation and the promotion of tumor survival. Studies on other related cancers have revealed some facts about EGFR roles. For example, increased levels of EGFR are linked to the increased proliferative and angiogenic activity of breast cancer cells. In addition, EGFR overexpression in prostate cancer cells reduces apoptosis through various mechanisms such as the Ras/Raf/MEK cascade and the Rac/PAK1 signaling pathway that are both involved in apoptosis. Therefore, EGFR has been reported as the most effective therapeutic target for CRC [14]. Despite the effectiveness of common monoclonal antibody-based drugs such as Cetuximab, Afatinib, Gefitinib, and Erlotinib which all target EGFR, their consumption is limited due to their high price. For instance, Cetuximab treatment costs about $80,000 a year. Therefore, scientists all over the world have tried to discover effective drugs with lower price.

EGFR receptors have also been shown to play their roles through binding to their targets; however, one of these receptors (ErbB-1) seems to internalize following ligand binding. Therefore, a large body of studies has focused on EGFR ability to produce a potent drug for cancer treatment. They also use such receptors to deliver nanoparticle and chemotherapeutic agents [15–18].

The present study was designed to evaluate the pro-apoptotic effect of the synthetic protein, called TGFaL3-SEB. This protein consists of two parts including the third loop (L3) of transforming growth factor-α (TGF-α) which binds to the staphylococcal enterotoxin type B (SEB) and Erb-1 with high affinity. Our observations indicated that TGFαL3-SEB may be a pro-apoptotic candidate for colon cancer treatment. However, further studies are required to clarify its potential role in cancer therapy.

2. Materials and methods

2.1. Cells and materials

E. coli TOP10 and BL21 strain (DE3) were purchased from Novagen Inc. (Madison, WI, USA). Human HT-29 and HEK-293 cells were provided from Pasteur Institute of Iran (Tehran, Iran). The Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and trypsin were procured from Gibco/Life Technologies (Carlsbad, CA, USA), while phosphate buffered saline (PBS), the 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide (MTT), penicillin-streptomycin and ethidium bromide were purchased from Sigma-Aldrich (MO, USA). Ni-charged chelating sepharose was obtained from Pharmacia Biotech (Uppsala, Sweden) and BCA was from Pierce (Rockford, USA). All antibodies were provided from Santa Cruz (CA, USA). Caspase activity assay kits were purchased from Biovision (California, US). The chemiluminescent detection system and cDNA synthesis kit were purchased from TaKaRa (Tokyo, Japan) and qPCR master mix and primers were provided from Bioneer (Daejeon, Korea). The rest of chemicals were obtained from Merck (Darmstadt, Germany).

2.2. Design of the TGFαL3-SEB chimeric protein

Recombinant TGFαL3-SEB sequence with KF445071.1 accession number were constructed by fusing the C-terminal of SEB and the N-terminal of TGFαL3 (TGFαL3-SEB) using hydrophobic GGSGSGGG amino acid linker as described in our previous study on pUC57 plasmid [1,2]. After verification of the construct's properties by Gen-Script (NJ, USA), the chimeric gene was synthesized by Shine Gene Molecular Biotech, Inc (Shanghai, China). The TGFαL3-SEB construct was dissolved in ddH_2O and then transformed into *E. coli* TOP10. After 24 h, the positive colonies of *E. coli* TOP10 were selected based on manufacturer's instruction.

2.3. Expression and purification of TGFαL3-SEB chimeric protein

The TGFαL3-SEB constructs were transformed into BL21 (DE3) and then induced with 0.5 mM IPTG overnight at 22 °C. The cell pellet was washed twice and then suspended in binding buffer (0.5 mol/l NaCl, 0.1 mol/l Tris-Cl, pH8.0), followed by a sonication step. The cell debris was washed and removed by centrifugation at 12,000 g for 30 min. The supernatant was exposed to a Ni-charged chelating agarose. After washing with 80 mM imidazole, the TGFαL3-SEB chimeric protein was eluted with the binding buffer containing 250 mM imidazole and then dialyzed against PBS overnight at room temperature (RT). TGFαL3-SEB chimeric protein was enriched by ultrafiltration and quantified by BCA assay as described previously. Finally, the purified proteins were confirmed by western blotting.

2.4. Cell culture

The HT-29 (human colorectal adenocarcinoma) and HEK-293(human embryonic kidney 293) cells were maintained in DMEM medium supplemented with 10% FBS and 1% PS (penicillin/streptomycin) antibiotics. The cells were cultured in T-25 flasks containing 5 ml of DMEM and incubated at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. The culture medium was changed every 48 h. The cells were seeded at a density of 1.5×10^4 cells/well in 96-well microtiter tissue culture plates prior to the TGFαL3-SEB treatment.

2.5. Cell proliferation assay

The MTT assay was performed for assessing the growth inhibitory activity of TGFαL3-SEB chimeric protein on HT-29 and Hek-293 cells as described previously [19]. This method is a colorimetric assay that measures the reduction of MTT by mitochondrial succinate dehydrogenase of viable cells. Briefly, the cells were seeded into 96-well plates at a density of 1.5×10^4 cells/well and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 until the adherent cells reached approximately 50% confluence. The DMEM was removed and the cells were serum-starved for 24 h prior to the treatment. The cells that were incubated in the culture medium were considered as the untreated cells and the rest of the cells were treated with various concentrations of TGFαL3-SEB chimeric protein for 24 h in a complete growth medium. Following such treatments, the medium was removed and 100 µl of MTT solution (5 mg/mL in sterile H_2O) was added to each well. The plates were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 4 h. After incubation, the MTT solution was removed and 200 µl aliquots of DMSO were added to each well followed by incubation at 37 °C for 10 min. The optical density (OD) of each well was read at 570 nm using an ELISA microplate reader (Bio-Rad, Hercules; CA, USA).

2.6. Cell ELISA assay

Binding of TGFαL3-SEB chimeric protein to EGFR was characterized by cell ELISA, based on a previous study [20]. In brief, 1.5×10^4 of cells per well were seeded into 96-well flat-bottomed plates. After 24 h, the cells were incubated with 25, 50 and 75 µg/ml of TGFαL3-SEB chimeric protein for 2 h and then the cells were fixed with 10% neutral formaldehyde at RT for 2 h. The cells were blocked by 5% bovine serum albumin (BSA) for 2 h. After washing cells three times with PBST

(10 mmol/l PBS pH7.4, 0.05% Tween-20), the cells were incubated with anti-hexahistidine mAb for 2 h, followed by another washing step and incubation with HRP-conjugated rabbit anti-mouse IgG. Finally, the color was developed by 0.4 mg/ml orthophenylenediamine (OPD) solution with 1.5% H_2O_2 and the absorbance at 490 nm was determined using an ELISA microplate reader (Bio-Rad, Hercules; CA, USA).

2.7. Flow cytometry detection of apoptosis using annexin V/ PI staining assay

Annexin-V binding and propidium iodide (PI) uptake was used to evaluate apoptosis. For this purpose, the treated and untreated cells were carefully scraped, separated by repeated pipetting and washed with PBS followed by flow cytometry. Analysis of quadrants is important to determine living cells (Annexin V-/PI-), early apoptotic/primary apoptotic cells (AnnexinV + /PI-), late apoptotic cells (Annexin V + /PI +) and necrotic cells (AnnexinV-/PI +). The cells were plated at a density of 1.5×10^4 cells per well into 24-well plates for 24 h and then were incubated with 25, 50 and 75 µg/ml of TGFαL3-SEB chimeric protein. The cells were then harvested and washed twice with PBS and suspended in 100 µl Annexin-V binding buffer followed by double staining with 10 µl FITC-labeled Annexin-V and 10 µl PI solutions (50 µg/ml in PBS). Ultimately, the samples were incubated at RT for 20 min and then analyzed by flow cytometry.

2.8. Caspase-3 and -9 activity assay

The treated cells were homogenized in ice-cold cell lysis buffer and kept at 4 °C for 1 h. The homogenates were then centrifuged at 12,000 g for 15 min at 4 °C. The supernatant was stored at −80 °C until use. Caspase-3 and -9 activity assays were performed using caspase activity colorimetric assay kit according to the manufacturer's instruction. Protein content was evaluated by using the BCA protein assay method [19]. The results of at least three independent experiments were expressed as fold change in caspase activity compared with untreated cells.

2.9. Analysis of gene expression by real-time quantitative PCR

The cells were seeded in 6-well plates and placed at 37 °C in a 5% CO_2 humidified incubator. The cells were treated with 25, 50 and 75 µg/ml of TGFαL3-SEB for 24 h in the complete growth medium. Total RNA was then extracted from cells using RNX-plus kit based on the manufacturer's instruction. Total RNA was reverse transcribed to cDNA using TaKaRa transcription kit, based on manufacturer's instruction and then quantitative real-time RT-PCR was performed. Briefly, the reaction mixture (10 µl) containing 2 µl of cDNA template, 1.5 µl each of forward and reverse primers and QuanTitect SYBR Green RT-PCR master mix amplified based on SYBR Green method. Each cycle of amplification was as follow: denaturation at 95 °C for 10 min and 35 cycles at 95 °C for 30S and 60 °C for 20S. Primers used in the present study were as follow; *bcl-2* forward: 5' CGA CTT CGC CGA GAT GTC CAG CCA G3'; *bcl-2* reverse :5'ACT TGT GGC CCA GAT AGG CAC CCA G3'; *bax* forward: 5'AGG GTT TCA TCC AGG ATC GAG CAG3'; *bax* reverse: 5'ATC TTC TTC CAG ATG GTG AGC GAG3'; *β-actin* forward: 5'TCA TGA AGA TCC TCA CCG AG3' and *β-actin* reverse: 5' TTG CCA ATG GTG ATG ACC TG3'. Finally, relative quantification normalized to the endogenous *β-actin* gene.

2.10. Statistical analysis

All examinations were set up in triplicates, repeated three times and the results were reported as the mean ± standard deviation (SD). The absorbance values obtained per treatment were converted to percentage of control cells. Statistical analyses were performed using version 20 and 19 of SPSS software (Chicago, IL, USA). The efficiency of the test sample was compared between treated and untreated cells by employing a Wilcoxon sign-rank test and one-way ANOVA. The $P < 0.05$ was considered as statistically significant.

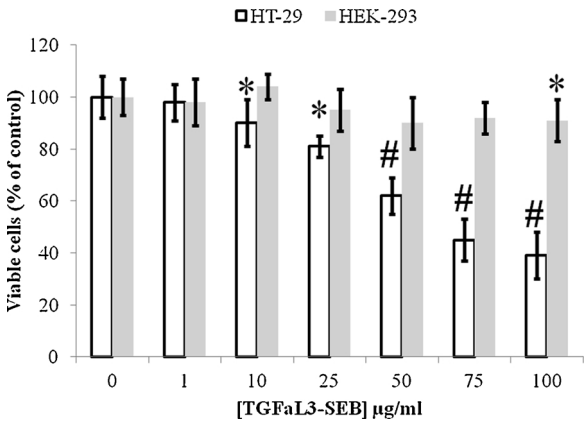

Fig. 1. Anti-proliferative effects of TGFαL3-SEB chimeric protein using MTT assay.
Cells were treated with different concentrations of synthetic TGFαL3-SEB protein for 24 h, each in triplicate. And then, cell viability was assessed by MTT assay. TGFαL3-SEB reduced the cell viability in a dose-dependent manner. Results (mean ± SD) were calculated as a percent of control values. *$p < 0.05$ and # $p < 0.001$ are significant. Statistical analysis was performed by ANOVA.

3. Results

3.1. TGFαL3-SEB chimeric protein produced by recombinant E. coli

Our previous study indicated that TGFαL3-SEB is a chimeric protein consisting of two parts that the third loop of TGF-α binds to SEB through a flexible hydrophobic GGSGSGGG amino acid linker. Further analysis in our previous study indicated that TGFαL3-SEB can bind to the cell surface of HT-29 which strongly express the EGFR protein [1,2].

3.2. TGFαL3-SEB chimeric protein decreased cell viability

As shown in Fig. 1, 24 h incubation of HT-29 cells with various concentrations of TGFαL3-SEB protein reduced cell viability, significantly. In detail, exposure of HT-29 cells to 1, 10, 25, 50, 75 and 100 µg/ml of TGFαL3-SEB protein for 24 h reduced the proliferation of control cells to 98, 90, 81, 62, 45 and 39%, respectively. As presented in Fig. 1, at the concentrations less than 25 µg/ml the cell proliferation did not reduce, significantly ($P < 0.05$). Therefore, the IC_{50} of TGFαL3-SEB protein treatment after 24 h was 61 µg/ml. However, this treatment was performed on HEK-293 cells, which had low expression of EGFR, but there was no difference between treated and untreated cells (100% versus 91 %). Our results revealed that only a concentration of 100 µg/ml of TGFαL3-SEB protein reduced the HEK-293 cells viability. In addition, this study led to further analysis of different concentrations of TGFαL3-SEB protein ranged from 25 to 75 µg/ml which exhibited significant effect on tumor cell ($P < 0.05$).

3.3. TGFαL3-SEB chimeric protein increased caspase activity

The caspase-3 activity was significantly higher after exposure of HT-29 cells to 25, 50 and 75 µg/ml of TGFαL3-SEB protein for 24 h compared to the control (110.3 %, 140.1%, and 160.6% versus 100%), but the caspase activity of HEK-293 cells which weakly expressed EGFR, was not different from that of the control. In addition caspase-9 exhibited the similar pattern of activity as caspase-3 (Fig. 2A). As presented in Fig. 2B, the caspase-9 activity was 115.8, 130.5 and 139.9% at the concentrations of 25, 50 and 75 µg/ml of TGFαL3-SEB protein after 24 h, respectively. Statistical analysis showed that there is a significant

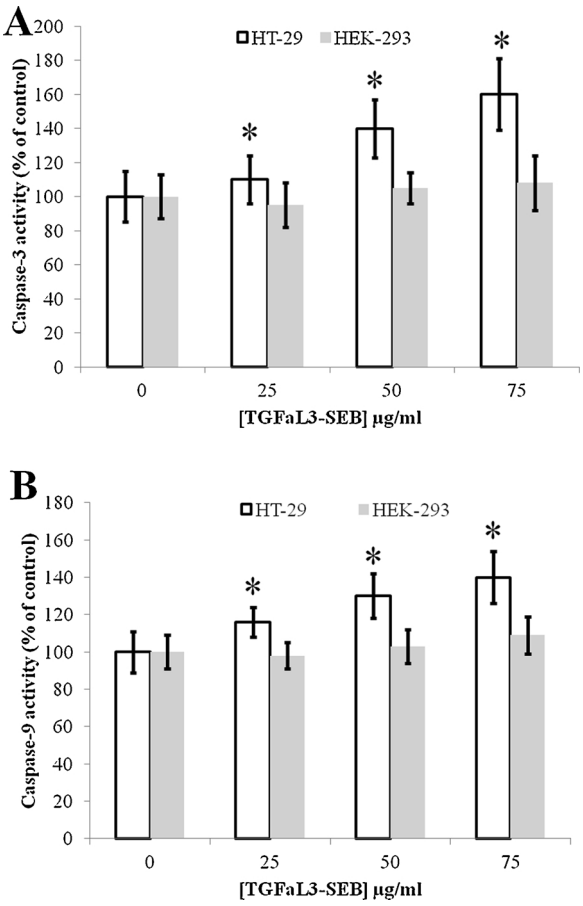

Fig. 2. Caspase activation in TGFαL3-SEB chimeric protein treated human colon HT-29 cells.
The cells were treated with the various concentrations of TGFαL3-SEB chimeric protein for 24 h and caspase activity was measured. A, in HT-29 cells, the caspase-3 activity was increased significantly in a dose-dependent manner, but HEK-293 cells exhibited no significant change in response to TGFαL3-SEB treatment. B, similarly the caspase-9 activity of HT-29 cells was increased, significantly. Data are means ± standard deviation of three independent experiments and reported as a percent of control cells (*$p < 0.05$ vs. untreated control).

difference between treatment groups versus control group ($P < 0.05$), however, HEK-293 cells treated with different concentration of TGFαL3-SEB protein for 24 h, exhibited no significant difference.

3.4. TGFαL3-SEB chimeric protein increased bax/bcl-2 ratio

It has been indicated earlier, that the *bax/bcl-2* ratio is one of the most important criteria used for identifying apoptotic cells [21,22]. In this study, our finding showed that the pro-apoptotic Bax mRNA expression level was significantly higher when HT-29 cells incubated with TGFαL3-SEB protein ($P < 0.05$). In this line, after 24 h incubation of cells with 0, 25, 50 and 75 µg/ml of TGFαL3-SEB protein, increased the Bax mRNA expression to 1.36, 1.63, and 1.82 fold change of control, respectively. However, Bax mRNA expression of HEK-293 cells did not change significantly, which might be due to the low expression of EGFR in HEK-293 cells (Fig. 3A). Conversely, the mRNA expression of *bcl-2* was decreased significantly ($P < 0.05$). As presented in Fig. 3B, *bcl-2* expression was 0.86, 0.77 and 0.72 fold after the treatment with 25, 50 and 75 µg/ml of TGFαL3-SEB protein for 24 h, respectively. Statistical analysis showed that there was a significant difference between treated and untreated cells ($P < 0.05$), however, the mRNA expression of TGFαL3-SEB protein did not change significantly after the exposure to

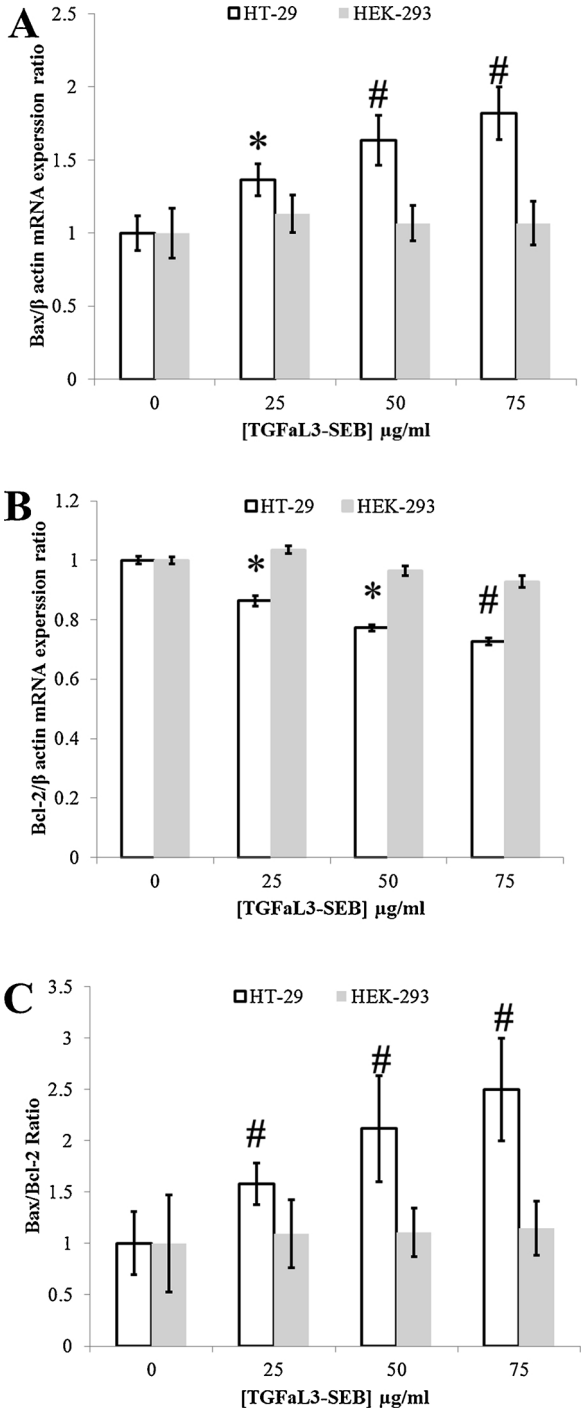

Fig. 3. Effects of TGFαL3-SEB chimeric protein on *bax*, *bcl-2* expressions and *bax/bcl2* ratio in colon cancer cells.
Cells were treated with different concentration of TGFαL3-SEB and then *bax* and *bcl-2* expressions were analyzed by real-time PCR. The data were average of three independent experiments and the relative expression values were calculated using the equation $RQ = 2^{-\Delta\Delta Ct}$ and reported as the mean ± SD. *$p < 0.05$ and # $p < 0.001$ are significantly different from control cells.

various concentration of TGFαL3-SEB protein for 24 h. Taken together, *bax/bcl-2* ratio of colon cancer HT-29 cells which highly express EGFR [23,24], significantly increased in a dose-dependent manner ($P < 0.001$), but this ratio in HEK-293 cells did not change prominently (Fig. 3C).

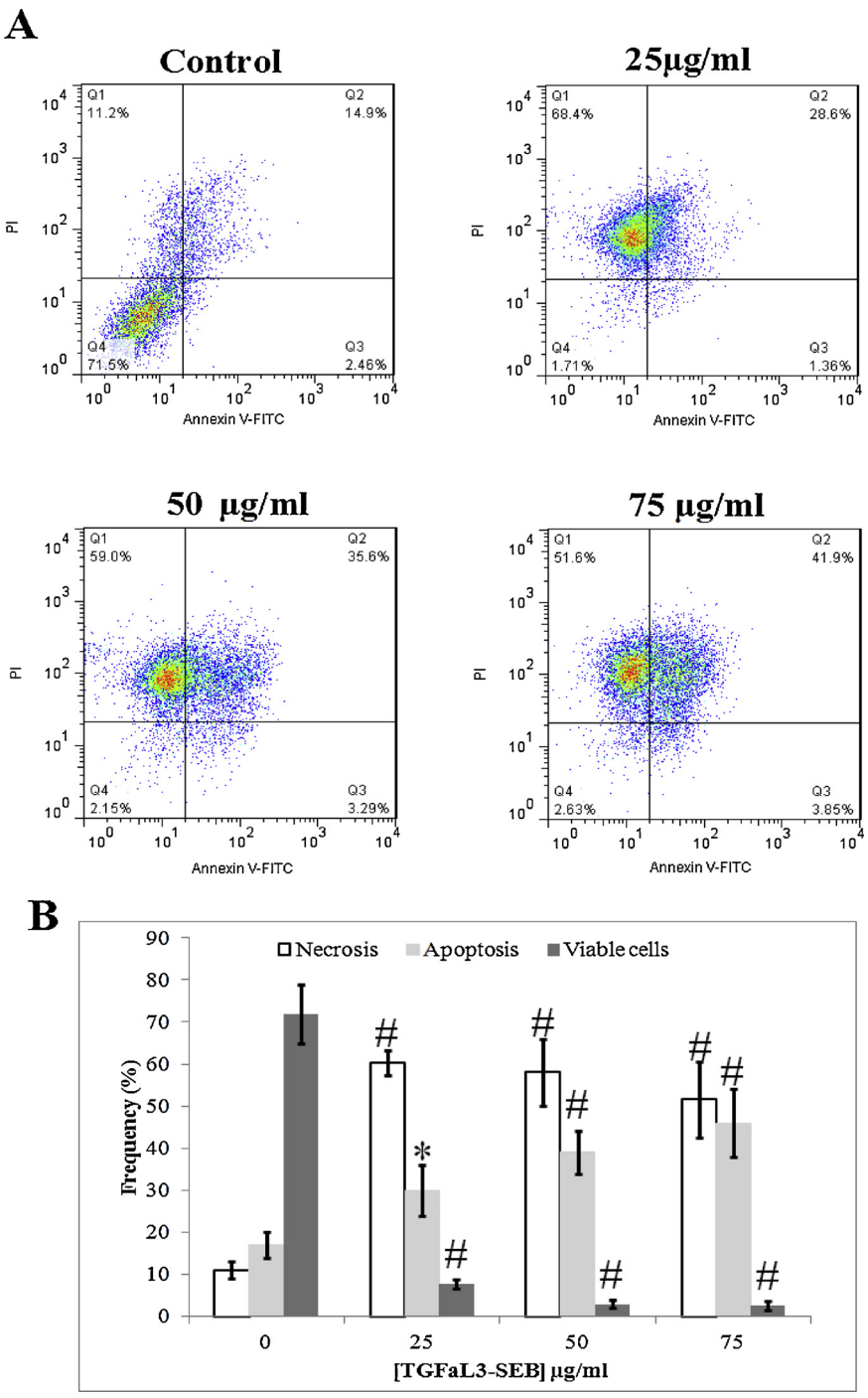

Fig. 4. Flow cytometry analysis of apoptosis induced by TGFαL3-SEB in colon cancer cell line.
Cells were treated with various concentrations of TGFαL3-SEB for 24 h and followed by apoptosis detection using annexin-V and propidium iodide (PI) by flow cytometry. A, the image represents the number of cells in each quarter. B, each value of the quarter was classified in three groups, including necrosis, early and late apoptosis and the viable cells. Date (mean ± SD) was calculated as a percent of total values. *$p < 0.05$ and #$p < 0.001$ are significant. Statistical analysis was performed by ANOVA.

3.5. TGFαL3-SEB chimeric protein induced apoptosis

To evaluate the growth inhibitory effect of TGFαL3-SEB and for assessing apoptotic cells, FITC-conjugated annexin V and PI double staining were used. Viable cells were negative for both annexin V-FITC and PI. Annexin V-FITC-positive/PI-negative cells were considered as early apoptotic, annexin V-FITC-positive/PI positive cells as late apoptotic and annexin V-FITC-negative/PI-positive cells as necrotic. In the current study, both early and late apoptotic cells were considered as the apoptotic cells. As reported in Fig. 4A, the treatment with TGFαL3-SEB led to increase the apoptotic cells in a concentration-dependent manner from 17% in control cells to 30, 39 and 46% in response to 25, 50 and 75 µg/ml of TGFαL3-SEB protein ($P < 0.05$). The percentage of necrotic cells were also increased significantly ($P < 0.001$) but was not concentration-dependent (Fig. 4B).

4. Discussion

The EGFR family consists of four cellular membrane receptors that have created an interacting system to receive and process information from cell environments in order to orchestrate normal processes, including growth, development and normal tissue turnover [25,26]. Today, it is generally believed that these receptors are more likely to be up-regulated in a wide variety of cancers, of which, colon cancer has been indicated to overexpress EGFR family members [27] and these receptors crosstalk to another signaling pathway, including insulin-like growth factors (IGF) and IGF-binding proteins [28–30]. EGFR receptors have been shown to play their role by binding to their targets. Recently, it has been reported that one of these receptors seems to internalized following ligand binding [31]. Therefore, this ability makes them a potential candidate for delivering specific cargo to cancer cells. On the other hand, it has also been indicated that ErbB-1 tyrosine kinase activity is necessary for ligand-dependent receptor shuttling and internalization [31,32]. Consequently, a large body of studies has focused on its ability to produce a potent drug for cancer treatment. Such receptors have also been used to deliver nanoparticle and chemotherapeutic agents to target cells [15–18] as in the current study, a ligand of the ErbB-1 receptor was fused to the staphylococcal enterotoxins B (SEB) to generate a potent agent.

Staphylococcal superantigens are a family of large enterotoxins that activate the immune system by binding to class II major histocompatibility complex without further antigen processing. SEB is one of the most potent staphylococcal enterotoxins which causes cellular cytotoxicity by inducing signaling cascade to involve inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 2 (IL2), IL-6, IL-10 and interferon-γ (IFN-γ). It has also been reported that SEB can activate chemokines such as monocyte chemoattractant protein 1 [33–35]. Moreover, it has been demonstrated that TNF-α plays a vital role in inflammation and T cell-mediated toxicity in SEB-induced septic shock [36]. Several, *In* vitro and in vivo studies confirmed that SEB may cause CD4 positive cells to induce cell death [37,38]. In order to clarify the underlying mechanism of apoptosis induction of SEB, Izquierdo et al. found that the inhibition of caspase activation pathways resulted in reduction of SEB-induced apoptosis [39]. Interestingly, further investigation showed that SEB can transcriptionally activate expression of some genes. For instance, it can induce the transcription of Rho family proteins in SEB-exposed renal proximal tubule [40].

Here, our finding indicated that produced chimeric protein selectively binds to HT-29 cells to highly express EGFR [23]. This binding to HT-29 cells was significantly higher than that for HEK-293 cells. Our data also confirmed that TGFαL3-SEB binding to its receptor was positively correlated with proliferation reduction of HT-29 cells. This fact might be due to inhibition of growth factor binding to EGFR. Similarly, the structural study revealed that Cetuximab interacts exclusively with domain III of EGFR, which sterically prevents the receptor from adopting the extended conformation required for dimerization and proliferation induction [41]. This finding confirmed that the exogenous antagonist of EGFR, like TGFαL3-SEB can prevent EGFR proliferative effects (Fig. 1). On the other hand, our finding also confirmed that TGFαL3-SEB can induce apoptosis and necrosis of exposed cells (Fig. 2 and 3).

5. Conclusion

Taken together, previous findings confirm the synthetic chimeric proteins which targeted EGFR exhibit extreme cytotoxicity and suggest that they have the potential to be a cost-effective alternative drug in the field of cancer treatment. In the current study, TGFαL3-SEB, chimeric protein was synthesized and its pro-apoptotic effect on EGFR expressing cancer cells (HT-29) was evaluated. Our data showed that treatment of cells with TGFαL3-SEB reduced cell proliferation, as indicated by cell proliferation assay and also induced apoptosis in HT-29 cell line, which was confirmed using caspase activity, *bax/bcl-2* ratio and flow cytometry. Our findings showed that the treatment of cells with TGFαL3-SEB protein can lead to the induction of apoptosis through the intrinsic pathway.

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Conflict of interest

No conflict of interest declared.

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