RESEARCH ARTICLE

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Investigation of Fibroblast Growth Factor Peptide Antagonist on Mouse Model Breast Tumor through *ERK/MAPK* and *PI3K/AKT* Signaling Pathways

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Abstract

Background: In the majority of cancers, metastasis of tumor cells is the main cause of treatment failure. This study intended to investigate the effectiveness of basic fibroblast growth factor (bFGF) peptide designed to inhibit tumor growth in 4T1 metastatic breast cancer through the *PI3K/AKT* and *ERK/MAPK* signal transduction pathways. **Methods:** The tumor was induced through 4T1 tumor graft in BALB/c mice. The designed peptide was injected intraperitoneal at three selected doses after two weeks for 14 days. The PBS and doxorubicin were used as the negative and positive control groups, respectively. Tumor size was measured and after the treatment period, the mice underwent a surgery and tumors were used for the western blot examinations. **Results:** the peptide injection was effective in reducing or inhibiting tumor growth in mice model and in vitro. The western blot analysis results showed that the p-AKT and p-ERK levels in peptide treated tumors were reduced ($p<0.05$). **Conclusion:** The peptide injection was effective in mice model. Findings showed that in the two signal transduction pathways, the p-AKT and p-ERK levels were significantly different from the negative control group.

Keywords: peptide- *ERK/MAPK, AKT/PI3K*- Breast cancer- tumor

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Introduction

Breast cancer is the most common malignancy and the major health-threating factor in women. According to statistics, breast cancer is the most prevalent malignancy and the malignancy leading cause of mortality among women [1]. Cancer is a multi-stage process including irreversible genetic changes in stem cells and, subsequently, clonal proliferation of these cells, and ultimately, development of an invasive phenotype and metastasis of cancer. Cancer prevention or treatment should be carried out at different stages of this process, using different phenotypes [2]. Today, the common treatments are surgery, chemotherapy, and radiotherapy [3]. In the majority of cancers, the metastasis of cancer cells is one of the main causes of treatment failure [4]. Although, surgery and radiotherapy are useful for local tumor treatment, they are not necessarily effective in dealing with metastatic cancers. In such cases, the treatment is often based on chemotherapy; however, due to the chemotherapy toxic side effects at high doses, chemotherapy has practically limited application. As a result, peptide therapy has become introduced as a widely used alternative treatment in the world [5]. Nowadays, the role of molecular pathways in cancer especially the pathways of immune system has attracted the clinicians because of their importance in diagnosis and treatment as clinical studies. Breast cancer as a solid organ malignancy is not an exception and observes the roles of pathogenesis such sustained angiogenesis [6]. By considering to the role of immune system in pathogenesis of breast cancer, this study investigated the effect of designed peptides of basic fibroblast growth factor (bFGF2) on growth of highly invasive and metastatic 4T1-MCT (mammary carcinoma tumor) as an animal model of breast cancer. Also, a highlighting prerequisite for tumor cell transport via the circulations and metastasis is believed to be tumor angiogenesis, and tumor angiogenesis inhibition has been revealed to be suppressed for tumor metastasis. In this experiment we aim to investigate whether bFG could also block the metastasis of malignant murine mammary tumor cells (4 T1 cells) or not.

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Materials and Methods

Synthetic peptide

The peptide was synthesis as describe earlier [7]. As glimpse the peptide synthesis and confirmed by using high-performance liquid chromatography, the peptide was synthesized and purified to a 90% purity, and then they were analyzed by the matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry, and ultimately through electrospray ionization mass spectrometry (ESI-MS).

A peptide was constructed with the sequence of CGGSGLPLGHIKC according to the sequence of basic fibroblast growth factor (bFGF2) [7]. The Shine Gene Biotechnologies Inc. (Shanghai, China) manufactured the peptide.

Metastatic breast cancer modeling and animals

Weak metastasis in mice can be induced by the human tumor cells, and unanticipated outcomes may ensue in case of metastasis occurrence. In spite of this, murine tumor renders more effective metastasis and features similar to those observable in cancerous patients [8]. Tumor in animals with normal immune system can be analyzed by conditions provided by this type of tumor. The pivotal contribution of immune system to the emergence and development of cancer [9] necessitates designing models for application in the perfect immune of mice to assess medicines and peptides. One of the several breast cancer cell lines is the 4T1 cell line, which is able to effectively induce metastasis to areas engaged in human breast cancer [10]. BALB/c mice are the laboratory bred strain of albino mice specially used for the study of cancer. Originated from BALB/c and the 4T1 breast cancer cell line was obtained from Pasteur Institute of Iran (Tehran, Iran). The cells were cultured in a glucose-rich DMEM culture medium with 10% fetal bovine serum and 5% inessential amino acids, as well as penicillin and streptomycin antibiotics incubated at 37ºC under 5% carbon dioxide environment. BALB/c was preserved at temperature of 24ºC, 12 hours light and 12 hours dark photoperiod, and sufficient moisture with adequate food and water for 5-7 weeks. The mice were retained in polycarbonate cages with lace doors.

Tumor implantation in animal model

Breast cancer was induced by two methods of 4T1 cell injection and cancer tissue transplant. In cancer cell injection procedure, the site of injection in rats was sterilized by cotton and alcohol. In day zero, all mice were hypodermically injected cancer cells (4T1; 1×106 cells/500µl or 1×10^5 cells/50 µl) at a place targeted next to the lowest right side breast gland. After 2 weeks the mice underwent euthanasia and surgery [7]. The Euthanasia defined as good death aiming at providing a quick, pain-free and non-stressful death. A fast unconsciousness is induced by carbon dioxide. The cage volume should be replaced by CO2 flow rate at 10% to 30% per minute. Then tumor cut into pieces of <0.3 cm^3, and subcutaneously implanted into the animal's right flanks under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia. Peptide treatment was applied upon reaching a tumor size of 0.5 to 1 mm3 after a 2-weeks period of tumor implantation. The tumor volume was measured every day by a digital Vernier caliper, using the following formula: volume = shortest diameter 2× Longest diameter× 0.52 [7].

The BALB/c mice having 4T1-MCT were then used to evaluate the antitumor activities of the peptide. To this end, rats were assigned at random to four groups of 10 mice each (n total = 40 mice).

Peptide Administration

The bFG antitumor efficacy was investigated in the murine 4T1-MCT model, and having built the solid tumors (tumor volume ~400mm^3), intraperitoneal (i.p.) administration of the peptide was done once a day at various doses of 1, 2.5, or 10 mg/kg over 14 days.

On day 28, the average volume of tumor in the different doses of bFG mg/kg, 2.5 mg/kg, and 10 mg/kg groups and phosphate buffered saline (PBS) treatment group were compared. There was not mortality during the experiments in any animal groups which experienced solid tumors, illustrating that bFG is not toxic at dosages used in this trial. In addition, we did not see any decrease in the animal's weight in bFG-treated groups; in fact, weight gain had been observed.

4T1 cells Viability analysis using MTT method

MTT is a method based on colorimetric method used to determine viability and cell toxicity of materials and is based on recovery of yellow soluble crystals of 4, 5 Dimethyl thiazole, 2-5 Diphenyl tetrazolium bromide (MTT) by Mitochondrial succinate dehydrogenase enzyme of living cells and formation of formazan purple solution crystals. Using measurement of optical absorption in 570nm using Ultraviolet-Visible Spectrophotometer and using standard curve, number of viable cells can be determined. The antagonistic property of the peptide was investigated on 4T1 cells in the presence of different concentrations of the peptide (50- 600 ng/ml) and 20 ng/ml of rhbFGF (Recombinant human basic fibroblast growth factor). 4T1 cells were seeded in 96-well plates with 5×10^3 cells per well. After well- plates were incubated under temperature of 37ºC and 5% CO_2, cells were washed with PBS and serum-starved in serum-free DMEM with 0.02 % FBS for 24. Cells were then treated with serially dilution peptides, 20 ng/ml bFGF for 24h. Then, the produced formazan was converted to solution through adding solvent like Dimethyl sulfoxide (DMSO). Finally, solution absorption was read in wavelength of 570 nm and number of viable cells was determined.

Immunohistochemistry

Due to immunohistochemical (IHC) analysis, excised tumor tissues were fixed in formalin (4%), embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H&E). Prior to immunohistochemistry (IHC), heat-induced epitope retrieval in a buffer at pH 9.0 was done.

To measure different proteins involved in various procedures such as CD31 and CD34 proteins to assign MVD (Microvessel density), Ki-67 in order to specify

the percentage of Ki67-positive cells, and also *P53* and *Bcl-2* to clarify the fraction of apoptotic cells relative to total cells, IHC staining was carried out followed by the formalin-fixed paraffin embedded sections.

Apoptosis Examination with Tunel Kit

Apoptosis was detected using the in situ Cell Death Detection Kit (Roche-11684817910). In brief, the sections were deparaffinized and dehydrated, and then rinsed with distilled water. The tissues were stored in a solution of proteinase K (20 mg/mL) at room temperature for 15 min. Endogenous peroxidase activity was also blocked through incubation in 3 mL/L solution of hydrogen peroxide/ methanol for 30 min at 37 °C. Sections were incubated with terminal deoxynucleotidyl transferase for 60 min at 37 °C. Then, dioxygenin-conjugated deoxyuridine triphosphate (dUTP) was added to the 3'-OH ends of the fragmented DNA molecule. Anti-dioxygenin peroxidase antibody was used to detect the labeled nucleotides. The sections were stained with diaminobenzidine and hematoxylin was used for background staining. The samples were analyzed using microscopy (Olympus BX-51, Japan), and having analyzed five random tissue samples under scale bars 100 and 20 µm, many positive cells were determined and quantitatively analyzed by ImageJ software.

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Western blot

In this study, the effects of peptide on *PI3K/AKT* and ERK/MAPK signaling pathways were investigated. To obtain homogeneous tumor tissue, approximately 150-200 mg of the tumor tissue was powdered in a mortar, lubricated in RIPA buffer (50 mL of Tris hydrochloride, 150 mM sodium chloride, 1% NP-40, sodium deoxy cholate, 10% sodium dodecyl sulfate, 0.01 M phenylmethylsulfonyl fluoride, emulsifiable EDTA) with a ratio of 1:5, and completely homogenized. The solution was then stored at 4°C for 30 min and then centrifuged at 14,000 rpm at 4°C for 15 min. The supernatant was removed and stored at -80°C. Protein concentration of specimens was determined using the Bradford method, based on the Bovine serum albumin.

To perform western blot, 20 µg of protein was loaded into each well, and the protein was isolated using the SDS PAGE (Sodium dodecyl sulfate Polyacrylamide gel electrophoresis) technique with 12.5% gel. The isolated proteins were transferred to PVDF (Polyvinylidene difluoride) membrane with a pore size of 0.45 µm then, the membrane was placed in a solution containing 50 mM Tris-HCl buffer, 0.1% TWEEN 20 Detergent, 150 mM NaCl, and 5% skimmed milk and stored for 1.5 hours. Then, the membrane was stored in an initial antibody solution with the concentration of 1 µg/mL overnight, diluted in a buffer containing 1% BSA and 2% skimmed milk. After rinsing the membrane, it was subjected to the secondary HRP to remove the non-attached antibodies and then rinsed again. Protein expression was measured using the ECL method. The membrane was exposed to the radiographic film and the bands appeared by processing the films in the darkroom. Then, the bands density was measured using densitometry.

Statistical analysis

The GraphPad Prism software (version 6.00) was used for data analysis, for the generation of graphs, and for statistical analysis. Data were provided as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was used to statistical significance for multiple comparisons and Two-way repeated measures ANOVA followed by Tukey's post-hoc test was used for therapeutic efficacy in affecting tumor growth. The $P<0.05$ was considered significant.

Results

Inhibition of tumor growth in mice model

To evaluate the antitumor effect of bFGF, inhibition of murine 4T1 cell line induced MCT growth were examined in BALB/c mice administration of the peptide in three doses 1, 2.5 and 10 mg/kg/day. The results obtained from peptide-treated samples were compared with each other and with PBS-treated control group. There was a statistically significant difference in antitumor activity of the bFGF groups and control group ($p<0.05$) (Figure 1).

Cell proliferation

The results showed that treatment of peptide for 24 h resulted in a dose-dependent decrease of 4T1 cell proliferation. Accordingly, the peptide interferes with the stimulatory effect of rhbFGF and act as its antagonist (Figure 2).

bFGF reduced cell growth, microvascular density, and induced apoptosis in the MCT mice model

To detect the therapeutic effect of bFGF and its powerful mechanisms behind that, we assessed microvascular density (MVD), tumor cell growth, and apoptosis changes by staining sections embedded in paraffin taken from 4T1-MCT (28 days after implantation) and examining them for possible changes related to the tumor cells. In breast tumors, MVD was utilized by the number of CD31+ and CD34+ stained microvessels in order to determine the bFG antiangiogenic features. Various dosages of bFGF (1 to 10 mg/kg) as treatment led to five and seven-fold reduction in the expression of CD31, and five and eight-fold decreases in the expression of CD34, in comparison with the PBS-treated control groups ($P<0.05$, $P<0.01$).

Immunohistochemical assessments also appeared that tumor cell proliferation can be inhibited by bFGF, as it is vividly shown by a 65% decrease in Ki67-positive cells compared to the control group ($P<0.01$). In peptide-treated mice group and within tumor tissue, to assess whether the defective tumor proliferation was associated with the promotion of apoptosis, MCT paraffin embedded sections were stained by TUNEL, which is an indicator of the late apoptosis, in addition to the anti-Bcl-2 and anti-*p53* antibodies as the early apoptosis biomarkers.

In peptide-treated mice, the percentage of TUNEL-positive cells was substantially higher than that in the control group ($P<0.001$). In fact, by *p53* and *Bcl-2* staining, the apoptogenic effect of bFGF was proved. Despite the fact that, in peptide-treated groups, a small

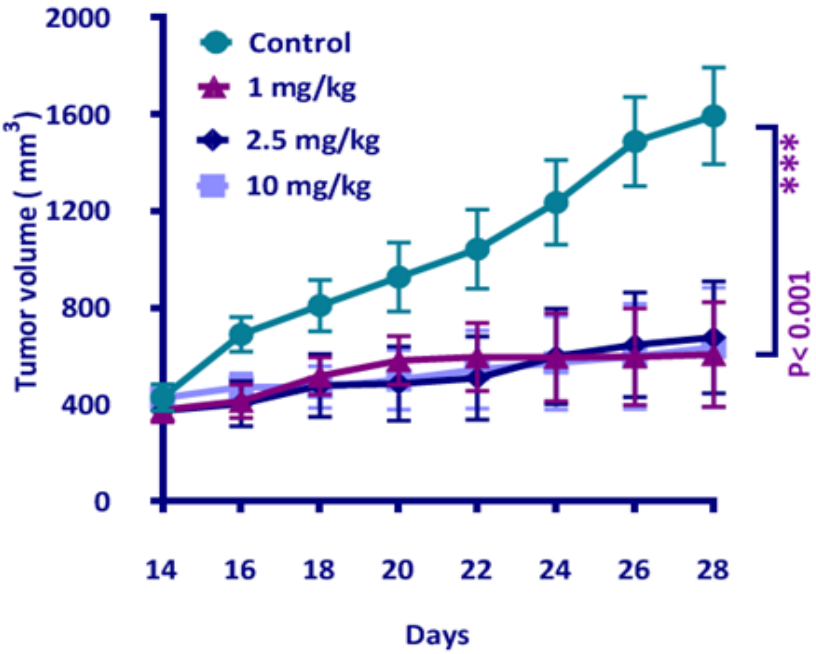

Figure 1. The Antitumor Effect of bFGF Peptide in Regression of 4T1-MCT. Mice (n=10) were ectopically implanted with 4T1 cells and treated with 1, 2.5, 10 mg/kg of the peptide for 14 days. Symbol *** denote examples of statistical significance.

number of cells were stained with anti-*Bcl-2* antibody, a significant number of *Bcl-2*-positive cells could be seen within tumors in the control group (P <0.001). Moreover, peptide-treated tumors, in comparison with PBS-treated groups exhibited significantly increased amount of *p53*-positive cells (P <0.001).

The histopathological experiments that we have done, indicated that bFGF considerably can inhibit tumor MVD and cell growth, and stimulated apoptosis in the tumor cells as well. In line with the modified cell proliferation and apoptotic changes, in the bFGF treated tumors compared with PBS-treated controls, H&E staining revealed potentially modified cell morphology. It is noteworthy to mention that, there were no significant differences in the immunohistochemical results for 1 and 10 mg/kg/day dosages (Figure 3).

Western blot for AKT and EKR expression

Expression of p-AKT (phosphorylated AKT), *AKT, p-ERK1/2* (phosphorylated EKR1/2), ERK1/2 levels in MCT treated with bFGF antagonist peptide. Relative levels of *p-AKT/AKT* and *p-ERK1/2/ERK1/2* were

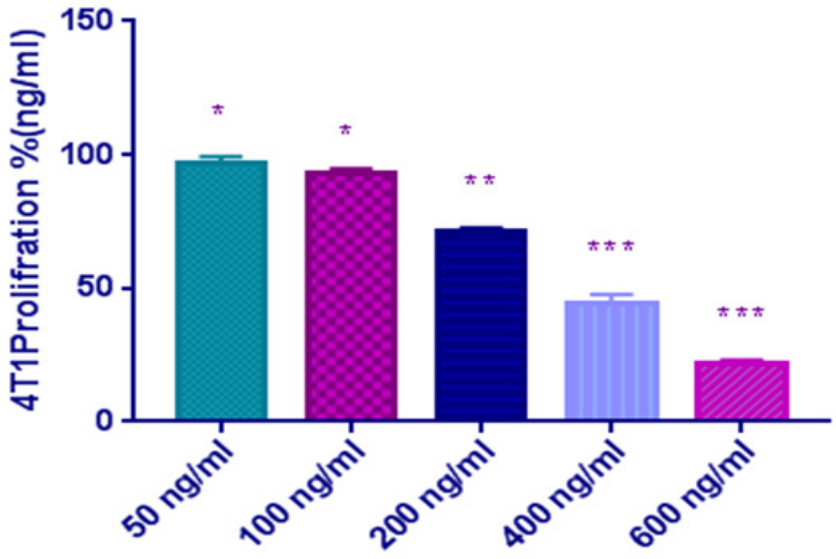

Figure 2. Cell Proliferation Graphics Determined by MTT assay after 24 hours Incubation with the Peptide (50- 600 ng/ml) and 20 ng/ml of rhbFGF. Symbol * denote examples of statistical significance.

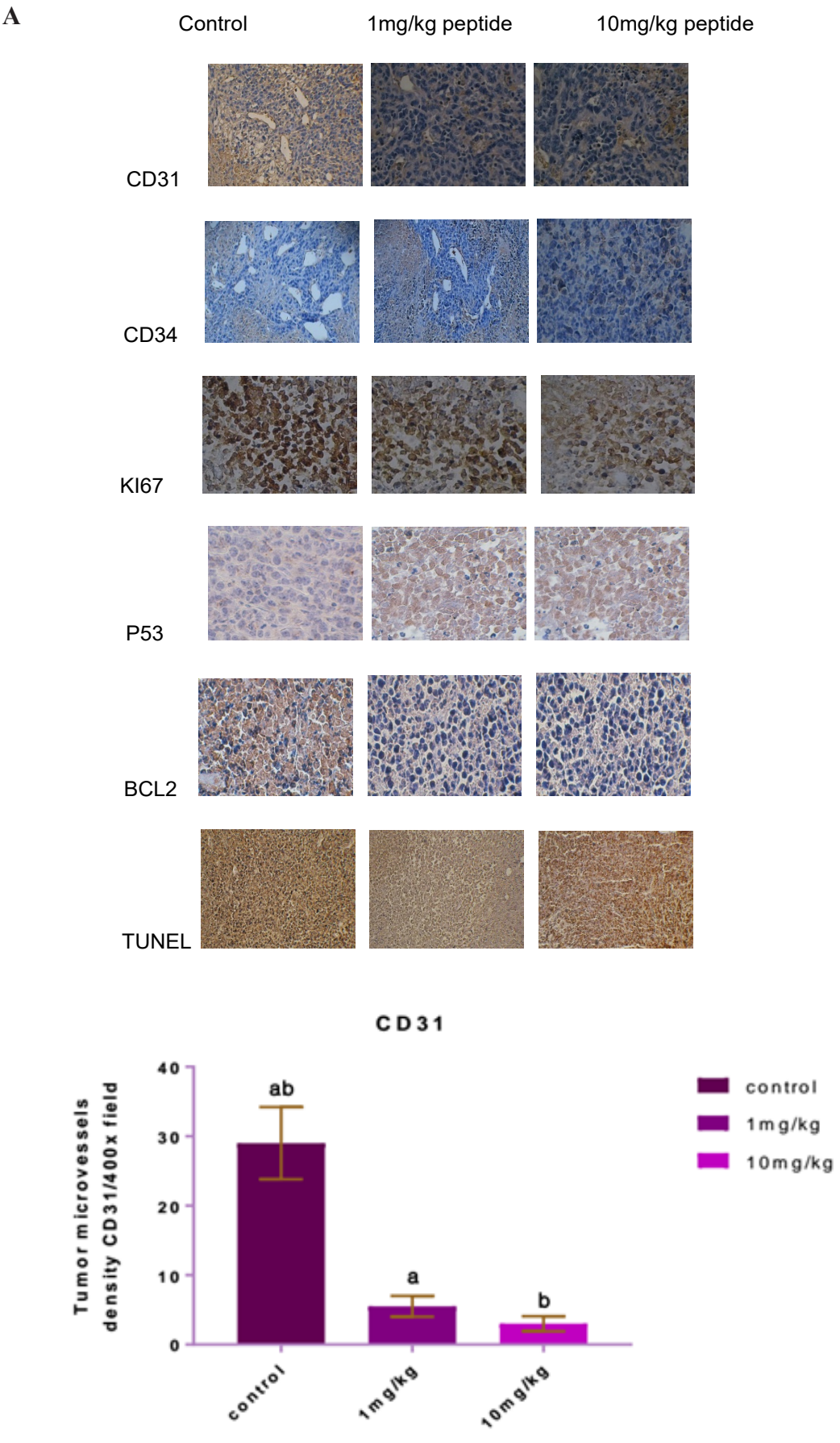

Figure 3. The bFGF Reduced Microvascular Density (A, B) Tumors were subjected to immunohistochemi staining. A representative picture has been shown for each treatment group in each case. TUNEL positive cells were calculated by number of positive (brown) cells ×100/total number of cells count in 10 randomly selected are as in each tumor sample (P≤0.05).

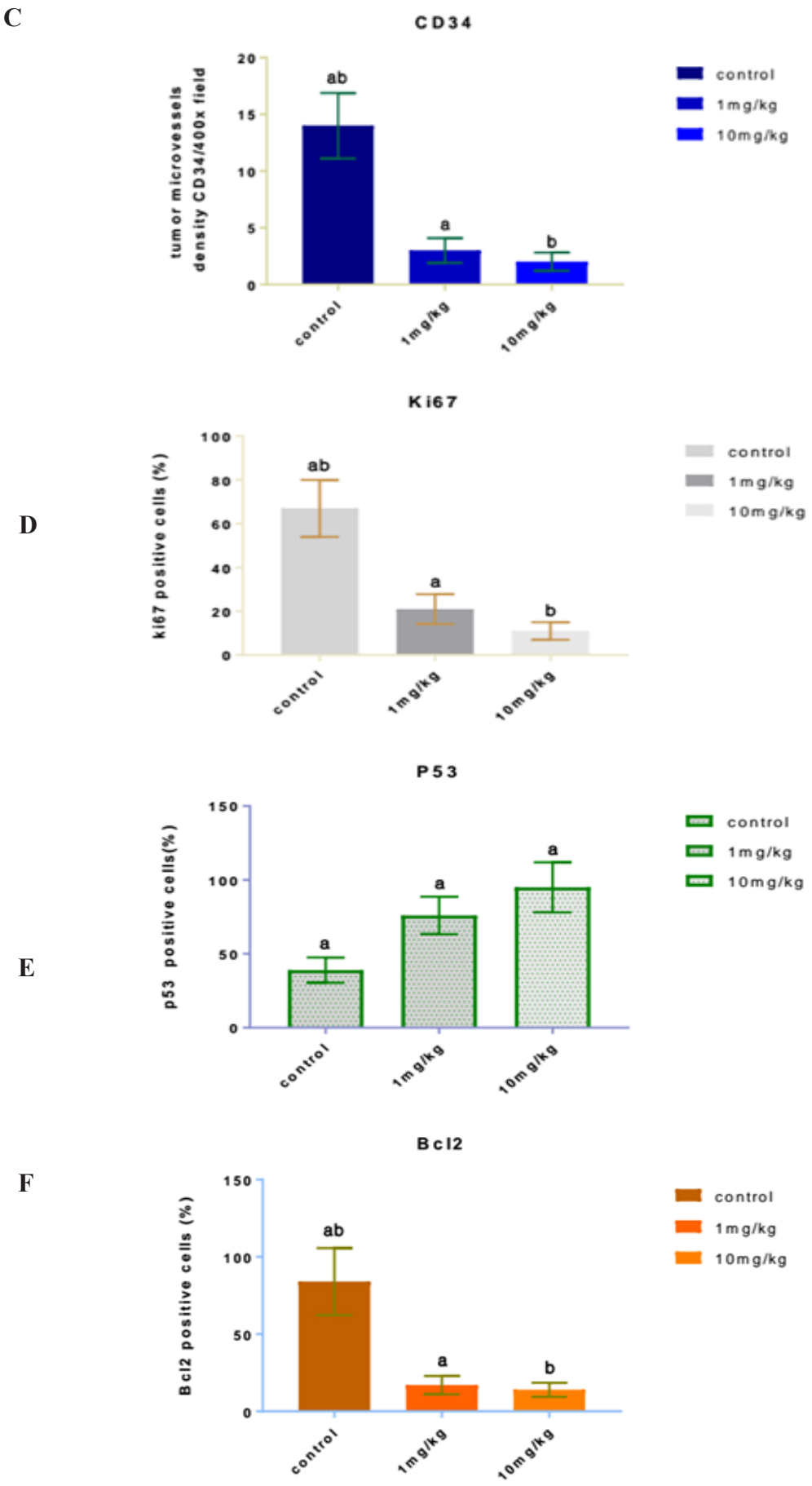

Figure 3. the bFGF reduced microvascular density (C), tumor cell growth (D) and induced apoptosis (E, F) in the MCT model. Tumors were subjected to immunohistochemi staining. A representative picture has been shown for each treatment group in each case. TUNEL positive cells were calculated by number of positive (brown) cells ×100/total number of cells count in 10 randomly selected are as in each tumor sample ($P \leq 0.05$).

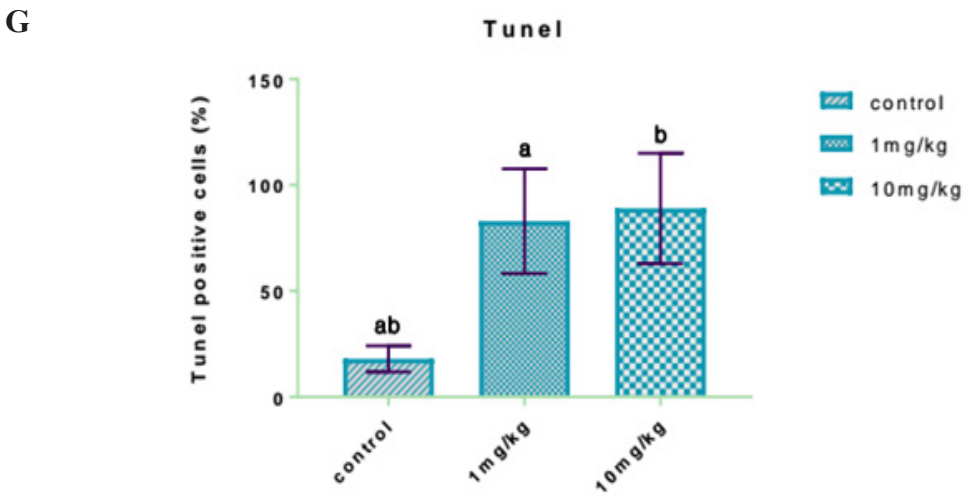

Figure 3. Induced apoptosis (G) in the MCT model. Tumors were subjected to immunohistochemi staining. A representative picture has been shown for each treatment group in each case. TUNEL positive cells were calculated by number of positive (brown) cells ×100/total number of cells count in 10 randomly selected are as in each tumor sample (P≤0.05).

evaluated by ImageJ. The results of Western blot showed that the engineered peptide significantly reduced the expression of the phosphorylated forms of ERK, AKT (P≤0.05). However, there was no significant change in the ERK and *AKT* expression level (Figure 4, Figure 5).

Discussion

Despite frequent studies, the exact mechanism for the antagonistic inhibitory and anti-cancer functions of peptides has not yet been identified. Peptide can affect the signal transduction pathways, including phosphoinositide 3-kinase (*PI3K*) and *AKT* (protein kinase B), and subsequently, the proliferation, differentiation, apoptosis, and metastasis processes [11-13]. Using different peptides that alter *PI3K/AKT* signaling pathways in cell lines for different cancers including Cholangiocarcinoma and melanoma were introduced earlier [14, 15].

The aim of this study was to investigate the effect of designed peptides of bFGF2 on growth of highly invasive and metastatic 4T1-MCT as an animal model of breast cancer. Also, evaluate the bFGF effect on the metastasis of malignant murine mammary tumor cells. In this regards, the study result indicates that, the bFGF could provide a regression in mice model and in vitro. Furthermore, the bFGF effects in MVD, tumor cell growth, and apoptosis changes were revealed. By the signaling pathway investigation, we show these effects of bFGF could be

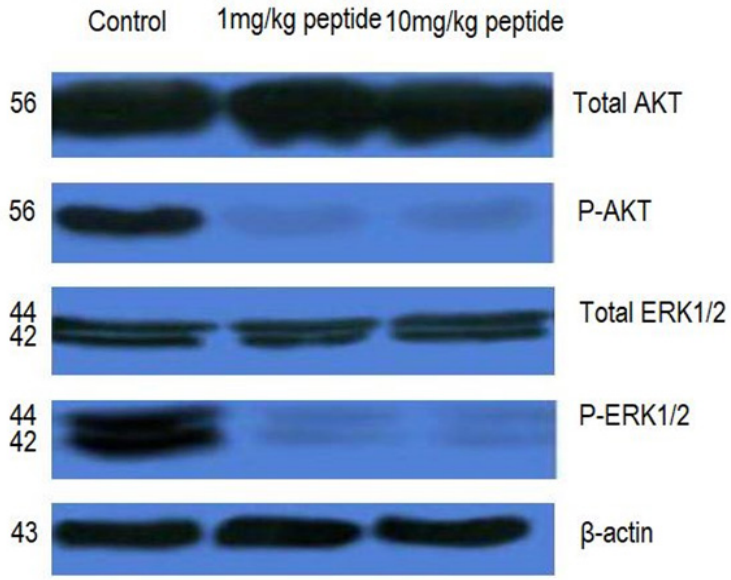

Figure 4. Western Blot Analysis was Done for *ERK, PERK, AKT, PAKT* Expression Levels together with β-actin as Loading Control from Tumors in each Control and 1mg/kg, 10 mg/kg Peptides Treatment Groups Detailed in Materials and Methods. In each case the western blot bands shown are from same. Relative levels of p-AKT/AKT and p-ERK1/2/ERK1/2 were evaluated by Image J.

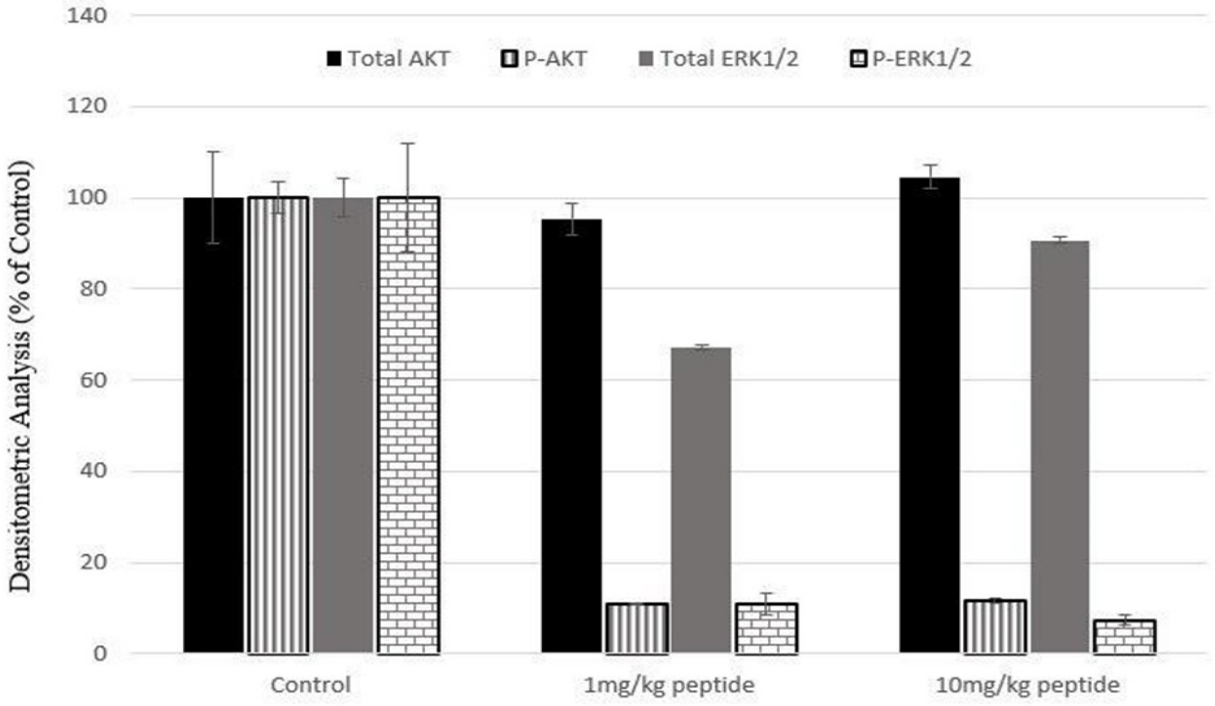

Figure 5. The Results Showed that the Engineered Peptide Significantly Reduced the Expression of the Phosphorylated forms of *ERK, AKT* (P≤0.05) and there was no significant change in the *ERK* and *AKT* expression level.

inducted due to the *AKT* and *EKR* expression alterations.

The *PI3K/AKT* pathway is involved in simultaneous control of metabolism, cell growth, and proliferation in healthy and malignant cells. In many types of cancers, the components of this pathway increase, and this is one of the most important reasons for increasing the survival rate and reducing the death of cancer cells [16, 17]. Activation of some cell surface receptors, including tyrosine kinase or G protein-coupled receptors, due to binding of ligands such as growth factors and insulin, leads to phosphorylation and activation of the PI3K enzyme and subsequent phosphorylation and activation of AKT as a central kinase in this pathway [16-18]. Active AKT leads to the phosphorylation of some compounds in the regulatory pathways of cell proliferation, differentiation, and survival, such as apoptotic proteins and transcription factors, which ultimately lead to inhibition of apoptosis and increased cell proliferation, growth, and survival [18].

The *MAPK/ERK* is a signaling pathway that begins with tyrosine kinase receptor ultimately reaching the DNA in the cell's nucleus and activating the genes involved in cell division. The *MAPK/ERK* pathway is connected to other pathways, such as *PI3K/AKT*, and activates this pathway. This pathway starts with tyrosine kinase membrane receptors of the ErbB EGFR family (fibroblast growth factor) and PDGFR (platelet-derived growth factor). After binding of a ligand to the receptor, the receptor forms a dimer and recalls Grb2 and SOS enzymes. These two enzymes convert GDP to GTP in the RAS enzyme. Once the RAS-GTP is formed, the enzyme is placed on the inner surface of the cellular membrane and the *MAPK/ERK* cascade is activated. The MAP Kinase proteins are phosphorylated and activated, which in turn activate parallel pathways, such as PI3K. It can also pass through the nucleus membrane and activate the transcription factors, such as G-myc, and cause the expression of the cell division gene. Erk induces epithelial cells through down-regulation of the Bim, a proapoptotic protein, which does not have the cytotoxic properties of Bax. The reduction of the Erk signal transduction pathway inactivates the integrin and reduces the expression of EGFR, which itself results in up-regulation and ultimately apoptosis [19, 20].

A compound activated by the *PI3K/AKT* pathway is the nuclear factor kappa β (NF-Kβ). This nuclear transcription factor is a heterodimer composed of P50-P65 proteins in the cell cytoplasm that enters the nucleus after activation and changes the gene expression. The enzymatic complex of the kinase inhibitor of kappa-β is one of the compounds phosphorylated by AKT, leading to NF-Kβ activation. The genes activated by this transcription factor play a role in cell cycle regulation, proliferation, survival, inflammation, and metastasis. Increased activity of these factors in cancer cells is one of the most important mechanisms of cell resistance to pharmacotherapy with ionizing radiation therapy [21-23]. The mTOR protein complex with its kinase activity plays a role in regulating cell growth, proliferation, survival, transcription, and protein synthesis. Increased expression and activity of this complex have been observed in many types of cancers. One of the activation pathways for this enzyme complex is its phosphorylation by phosphorylated of AKT, due to the *PI3K/AKT* pathway activity [24, 25].

The current cancer treatment strategies have been

followed by many complications, such as damage to healthy cells and drug resistance, resulting in its recurrence. As a result, it is essential to employ new therapeutic approaches with stronger effects and weaker toxicity and fewer adverse effects, such as peptide therapy. Folkman [26] was among the leading researchers who recommended the inhibition of tumor blood vessel formation for cancer treatment. Regarding the high selectivity and low cost of peptides, these compounds as a highly suitable candidate with fewer side effects and lower toxicity [27]. Khumalo et al. [28] supported discovery and identification of new methods for cancer treatment to increase sensitivity of cancer cells, since cancer resistance to conventional treatments have increased. The first FDA-Approved anti-angiogenic drug was reported by Eichhorn et al. [29] which could be used to treat solid tumors and inhibited endothelia growth factor. Stupp et al. [30] reported that combined chemotherapy can show synergistic effects in cancer treatment. Anderson et al. [31] stated that combination of chemotherapy and radiotherapy would present synergic effects. In 2012, Li et al. [32] introduced a peptide with the ability to not only inhibit bFGF proliferation in cancer cells of breast, but also induce cell-cycle arrest in G0/G1 phase of cell cycle. Macrovich et al. [33] suggested the clinicians to make use of new techniques for drug delivery as well as development of methods with synergistic effects besides application of antiangiogenic treatment modalities in preclinical animal studies [33].

By considering all these, the results of our current study are in the direction of the approving of anti-tumor effects of the peptides, the bFGF for cancer treatment in animal models. The major limitation in our current study was in limited number of animal models. Further study, with greatest in animal number could provide a better understanding of the dose of the bFGF. Furthermore, another limitation in our current study could be the transcriptome analysis for better and clearer understanding of the molecular mechanism of the bFGF.

Antiangiogenic therapies are popular approach in cancer treatment; Cell proliferation, survival, invasion, and metastasis are hallmarks of carcinogenic process. Signal transduction alteration can lead to uncontrolled cell proliferation. Several signaling pathways are vital to maintain normal human physiology including phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and the Raf/mitogen-activated and extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK). These signaling pathways are dysregulated in many human cancers such as breast cancer. *PI3K/AKT*/mTOR and Raf/*MEK/ERK* cascades are interrelated with cross-talks and several convergence points, and feedback loops which is proven by both in vitro and in vivo data. Mutations in Raf/*MEK/ERK* and *PI3K/AKT*/mTOR pathways may be present simultaneously. Signaling may be maintained in case of one pathway inhibition; by means of signaling through the reciprocal pathway. Many studies are already underway aiming either one of the pathways or both pathways in patients with breast cancer. Considering the critical physiological role of *PI3K/AKT*/mTOR and Raf/*MEK/ERK* signaling pathways, monitoring of toxicity profile of dual pathway targeting in of great importance [34]. Key role of ERK1/2 signaling in proliferation induction is well known as well as its role in development and progression of cancer. Consequently, it is of no surprise that ERK1/2 cascade inhibitors such as Vemurafenib and Trametinib [35, 36] are considered among anticancer drugs. ERK1/2 cascade, as an intracellular signaling pathway, includes sequential phosphorylation and activation of Raf or other MA3Ks, MEK1/2, ERK1/2 and many downstream MAPKAPKs. This cascade is responsible for many cellular process, especially cell proliferation and differentiation [37-39]. Dysregulation of this pathway, as a central regulatory pathway, is known to be play role in cancer pathophysiology [40, 41]. In 2018, Plotnikov et al. [42] reported a proof of concept that ERK1/2 nuclear translocation may be targeted in treatment of various cancers related to ERK1/2.

In conclusion, this study investigated the effect of designed peptides on growth of highly invasive and metastatic 4T1 MCT. Results indicated regression of tumor growth. The difference in this reduction was significant between the negative control groups with peptide groups. Our results indicated the effectiveness of peptide therapy and showed that the P-AKT and p-ERK levels in tumors treated with peptide reduced in two mentioned signaling pathways.

Author Contribution Statement

MJ, SG, AT designed and performed the experiments, participated in the interpretation of data, and wrote manuscript. SG, HR performed the biological experiments, interpreted the data, and wrote the manuscript. All the authors SG, AT, HR, MJ read and approved the final manuscript.

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General

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Availability of data

All data generated or analyzed during this study are included in this published article or available from the corresponding author on reasonable request.

Ethical Declaration

This research was carried out in accordance with the ethical guidelines of research on the experimental animals of the National Ethics Committee in Biomedical Research of the Islamic Republic of Iran and Iran University of Medical Sciences. All protocols were approved by the committee.

Competing interests

The author declares that he has no competing interests.

Funding statement

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