Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Pomegranate peel extract inhibits expression of β-catenin, epithelial mesenchymal transition, and metastasis in triple negative breast cancer cells

Mehdi Bagheri[1], Mozhgan Fazli[2], Sara Saeednia[2], Aylar Kor[3], Naghmeh Ahmadiankia[2,4*]

[1] Clinical Research Development Unit, Imam Hossein Hospital, Shahroud University of Medical Sciences, Shahroud, Iran
[2] School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
[3] Student Research Committee, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
[4] Cancer Prevention Research Center, Shahroud University of Medical Sciences, Shahroud, Iran

Correspondence to: Ahmadian@shmu.ac.ir

Received November 30, 2017; **Accepted** May 29, 2018; **Published** May 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.7.15

Abstract: The standard treatment for triple-negative breast cancer (TNBC) is chemotherapy, which is highly toxic to patients; thereby, there is a need to identify safer and more effective therapeutic approaches. Medicinal plants constitute a common alternative for cancer treatment. Pomegranate is a well-known fruit in this context, but its antimetastatic property has not been extensively studied. As breast cancer-related deaths from TNBC are mainly due to metastasis, the present study was designed to investigate the antimigratory effect of pomegranate peel extract (PPE) on TNBC cells. For this purpose, the MDA-MB-231 cells were treated with different concentrations of PPE for 24, 48 and 72 hr. The effects of PPE on cell migration and invasion were determined by wound healing and transwell assays. To address the possible molecular mechanisms underlying the antimetastatic effect of PPE, real-time quantitative PCR analysis of selected epithelial mesenchymal transition (EMT) markers were performed. Moreover, the expression of β-catenin as a critical factor in promoting cancer metastasis was examined. PPE markedly inhibited the migration and invasion of cells at concentrations of 25, 50, 100, 250, 500, and 1000µg/ml. At relatively high concentrations (500, 1000µg/ml), PPE induced apoptosis. Moreover, PPE decreased the gene expression of vimentin, ZEB1, and β-catenin and also increased the expression of E-cadherin in TNBC cells. The protein level of β-catenin, as measured using western analysis, revealed a time-dependent decrease at the concentration of 1000µg/ml PPE. Downregulation of EMT markers and β-catenin showed accordance with the inhibition of migration and invasion. The present data show that PPE could be a promising drug candidate to reduce metastasis in TNBC cells.

Key words: Pomegranate peel extract (PPE), triple negative breast cancer (TNBC), metastasis, epithelial mesenchymal transition (EMT), β-catenin.

Introduction

Triple negative breast cancer (TNBC), in which cells do not express estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), is a highly aggressive breast cancer subtype, responsible for 10%–20% of breast cancers (1). Since TNBC lacks treatment targets due to its triple-negative nature, chemotherapy is presently the only available standard treatment for it (2). However, chemotherapy has a wide range of short and long-term side effects which result in changes in the quality of life of patients (3). Furthermore, patients suffering from TNBC often display chemotherapy resistance which makes the treatment difficult for them (4). In TNBC cases, distant metastasis is frequent due to the development of drug-resistant cancer cells (5). The limited efficacy of current therapies against TNBC tumors leads to search for novel combinatorial treatments. Recently, there has been an interest to use medicinal plants to fight cancer (6). Among them, pomegranate *(Punica granatum, L.)* is valued for its high content of polyphenols including ellagic acid, ellagitannins, and other flavonoids (7). Pomegranate native to Iran has dark red peel which represents the presence of excess phenolic and flavonoid contents (8,9). Despite the highest antioxidant activity that is in line with high content of polyphenols in the peel, the literature regarding the anticancer potential of pomegranate is mainly focused on the fruit juice or seed and very little data is available on pomegranate peel extract (PPE). Moreover, the most recent studies involving PPE mainly focus on its antioxidant and antiproliferative effects (9, 10). The work presented here, goes beyond these findings by evaluating the antimetastatic effect of PPE on TNBC cells. As epithelial mesenchymal transition (EMT) is involved in invasion and metastasis of tumors, the second objective of this study was designed to find out whether PPE treatment can change the expression of some key elements of EMT including vimentin, ZEB1, and E-cadherin in MDA-MB-231 cells as a subtype of TNBC cells. Moreover, upregulation of Wnt/β-catenin signaling plays a pivotal role in regulation of cell migration (11). Accordingly, to address whether β-catenin pathway is impaired following PPE treatment, the expression of β-catenin in PPE-treated TNBC cells was determined. Understanding the mechanism of PPE action and identification of its targets provide a rational basis for the potential clinical application of PPE in preventing breast cancer metastasis.

Materials and Methods

Preparation of extracts

Pomegranate fruits were collected from Khorasan province, Iran in 2016. The peel parts were separated, dried and grounded into fine powder. Extractions were performed with methanol. The extracts were concentrated by rotary evaporator and then dried in very low pressure. The dried extracts were stored at -20°C.

Cell culture

Human breast cancer cell line MDA-MB-231 was obtained from Pasteur Institute (Tehran, Iran). Cells were cultured in medium containing DMEM supplemented with 10% FBS and 1% penicillin- streptomycin (10000 units/ml) as antibiotics. Cells were incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

Wound healing assay

The effect of PPE treatment on cell migration was determined using wound healing assay as described previously (12). Briefly, a fine scratch was made on the surface of monolayer culture when the cells were approximately 80% confluent. A cell free area of approximate 1 mm in extent was generated. Micrograph images from scratch zone were taken at time=0 h. Then cells were incubated with 12.5, 25, 50, 100, 250, 500 and 1000 µg/ml of PPE. The choice of these doses was based on previous study (9). Other micrographs were taken from the same region, after 24, 48 and, 72h. The gap width of scratch was compared with the gap size at 0 h and analysed by the Image J software.

Transwell migration assay

5×10^4 cells in 400µl culture medium without serum were plated on the upper side of polycarbonate membrane (8 µm pore size) of transwell (SPL Life Sciences, Korea). The transwells were introduced into 24-well plates and 800 µl of medium composed of 10% FBS was added to the lower chamber. Then cells were incubated with 12.5, 25, 50, 100, 250, 500 and 1000 µg/ml of PPE and allowed to migrate for 24 h. The cells on the upper side of the membranes were removed, and then the membranes were fixed in methanol 100% and stained with 0.1 mg/ml DAPI (Sigma). Cells were counted in four fields (10X) for each sample by fluorescent microscope (IX71; Olympus) to obtain the average number of cells that migrate from the top of the membrane to the underside.

Transwell invasion assay

Cell invasion was performed using precoated invasion chambers with 8 µm pore size membrane (SPL Life Sciences, Korea). MDA-MB-231 cells (5×10^4 cells) treated with different concentrations of PPE (0, 12.5, 25, 50, 100, 250, 500, 1000 µg/ml) in 400 µl medium without FBS were seeded onto the upper chamber. A volume of 800 µl DMEM medium with 10% FBS was added to the lower chamber. The filter inserts were removed from the wells after 24 h incubation at 37°C. The non-invading cells on the top side of the filter were removed gently with a cotton swab. The invaded cells on the bottom of the filter were fixed with 100% methanol for 20 min at −20°C, and then stained with 0.1 mg/ml DAPI (Sigma). Four vision fields were selected randomly per well under a fluorescent microscope and the number of cells that penetrated the membrane was counted.

Apoptosis assay

The control and PPE treated cells were suspended in 500µl of 1X binding buffer. Then, cells were incubated with 5µl of annexin V-FITC and 5µl of propidium iodide (PI) in dark for 15 min at RT. Quantification of Annexin-V/propidium iodide incorporation was performed using a FACScalibur flow cytometer (BD Biosciences, San Jose, CA, USA). Early and late apoptotic cells and also necrotic cell populations were visualized by constructing a dot-plot with the aid of FACS. The FL1 channel was used to detect annexin-V FITC staining and the FL2 channel was used for PI staining. The data were analyzed using the Win-MDI software.

RNA extraction and real-time PCR

By using TriPure isolation reagent (Roche, Germany) based on the manufacturer's instructions, total RNA was extracted from cells. The extracted RNAs were treated with DNase I enzyme to eliminate probable contamination with genomic DNA. Reverse transcription and real-time PCR were performed following the manufacturer's instructions using SYBR Green master mix (Parstoos, Iran) with primers for GAPDH (forward 5'-AAGGTGAAGGTCGGAGTCAAC-3' and reverse 5'-GGGGTCATTGATGGCAACAATA- 3'); β-catenin (forward 5'-AAAATGGCAGTGCGTTTAG-3' and reverse 5'-TTTGAAGGCAGTCTGTCGTA-3'); vimentin (forward 5'-ACCCGCACCAACGAGAAGGT-3' and reverse 5'-ATTCTGCTGCTCCAGGAAGCG-3'); ZEB1 (forward 5'-TGCACTGAGTGTGGAAAAGC-3' and reverse 5'-TGGTGATGCTGAAAGAGACG-3'), and E-cadherin (forward 5'-TTGCACCGGTCGACAAAGGAC-3' and reverse 5'- TGGATTCCAGAAACGGAGGCC-3'). Amplification was carried out using 40 cycles of PCR in CFX96 Real-Time PCR Detection System (Bio-Rad), with the following program: denaturation at 95˚C for 10 min and 40 cycles of 95˚C for 15 sec, 60˚C for 30 sec, and 72˚C for 30 sec (13). The housekeeping gene, GAPDH was used to normalize target gene expression. The mRNA comparative expression level of each target gene was calculated by $2^{-\Delta\Delta CT}$. In addition, melting curves were used to determine non-specific amplification.

Western blotting analysis

The control and treated cell pellets were suspended in lysis buffer (BioBasic, PH= 8), supplemented with protease inhibitor cocktail (Sigma-Aldrich). Forty micrograms of proteins were denatured using Laemmli's sample Buffer and boiled at 95 °C for 5 min. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred onto PVDF membranes (Amersham). The membranes were placed in a blocking buffer for 60 min at RT and subsequently incubated with primary anti-β-catenin antibody (1:1000; Cell Signaling) overnight, at 4°C. The following day, the membranes were washed and incubated with a peroxidase conjugate secondary antibody (1:5000; Cell Signaling) for

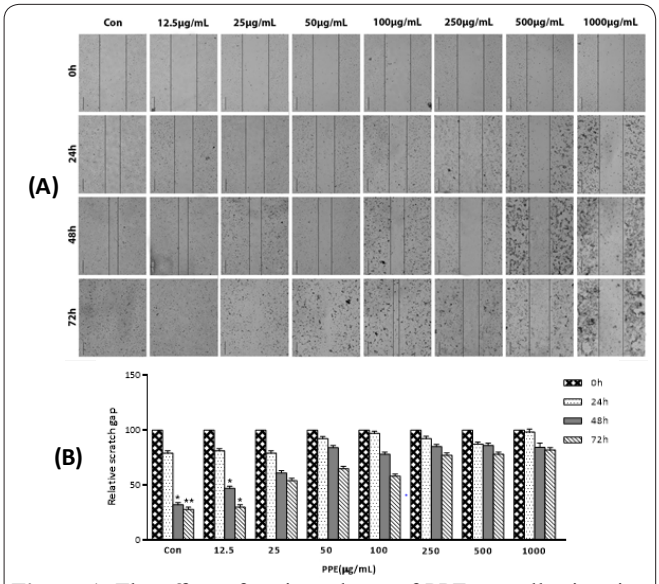

Figure 1. The effect of various doses of PPE on cell migration was examined by wound healing assay. (A) Wound healing images show the extent of healing and black lines indicate the wound borders. Scale bar: 200 µm. (B) Statistical analysis of the wound healing assay. * and ** donate P <0.05 and P <0.01, respectively, as compared to the control.

60 min, at RT. Then membranes were incubated with the Bio-Rad Clarity™ western ECL substrate and finally exposed to X-ray films from FUJIFILM Corporation. Band intensity was calculated using the Image J software.

Statistical analysis

All data were subject to statistical analysis using GraphPad Prism version 6.0. The significance of difference between the experimental groups and controls was assessed by one-way ANOVA followed by dunnett's test. Data are presented as mean ± standard deviation (SD).

Results

PPE suppressed migration of breast cancer cells

To investigate the inhibitory effect of PPE on migration of MDA-MB-231 cells, the wound healing assay was performed. The results demonstrated that PPE suppressed migration of cells to the denuded zone as early as 24h after treatment, which continued more than 72h, and the effect was dose-dependent. PPE inhibited cell migration even at relatively low doses (Figure 1A, B).

To confirm the effect of PPE on cell migration, we did transwell migration assay. As shown in figure 2A, B, the number of migrated cells decreased in a dosage-dependent manner as early as 24 h after treatment.

PPE inhibited invasion via a dose-dependent manner

To determine whether PPE weakens the cell invasive potential, the transwell invasion assay was performed and the invaded cells were counted. The results showed that treatment with PPE (50, 100, 250, 500, 1000 µg/ml) for 24 h significantly decreased the number of invading cells versus control in a dose-dependent manner (Figure 3).

PPE induced cell apoptosis in MDA-MB-231 cells

We investigated the apoptotic effect of PPE on MDA-MB-231 cells to rule out the apoptotic impact of PPE on migration assay results. As shown in figure 4, PPE

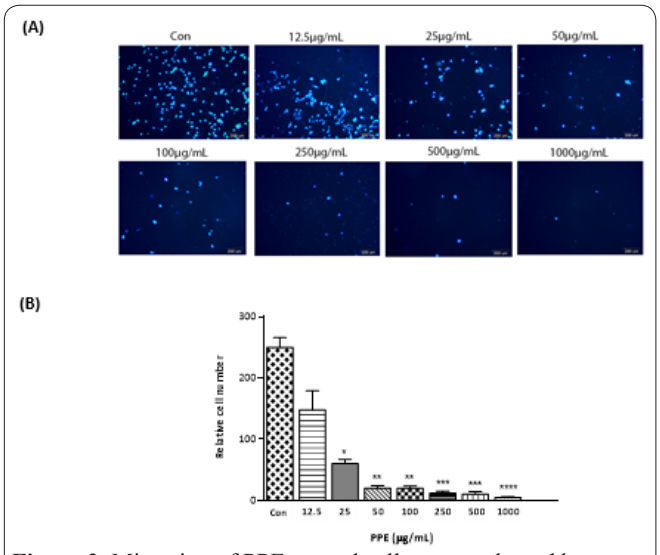

Figure 2. Migration of PPE treated cells was evaluated by transwell migration assay. (A) Representative images of migrated cells stained with DAPI. (B) The results presented are an average number of migrated cells on the underside of the filter. Significance was set at *P< 0.05, **P< 0.01, ***P< 0.001, ****P< 0.0001.

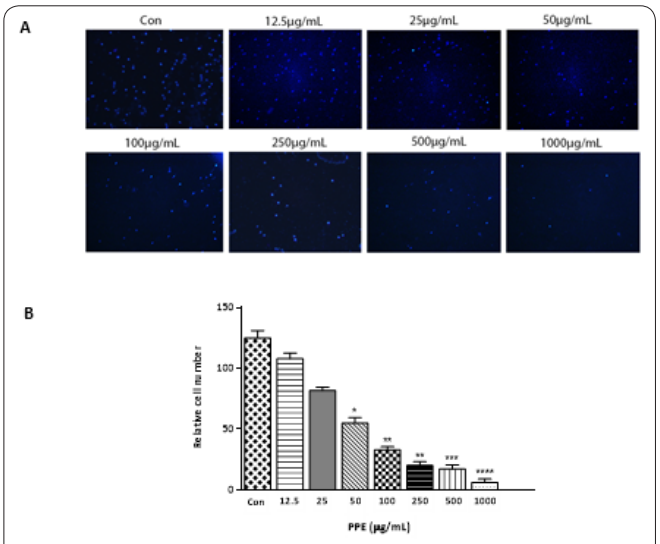

Figure 3. PPE inhibited invasion. (A) Representative images of invaded cells stained with DAPI. (B) Statistical analysis of total invasion percentage. The bar chart showed that the number of invaded cells was significantly lower in PPE-treated MDA-MB-231 cells (50, 100, 250, 500, 1000 µg/ml) than that of control cells. Significance was set at *P< 0.05, **P< 0.01, ***P< 0.001, ****P< 0.0001.

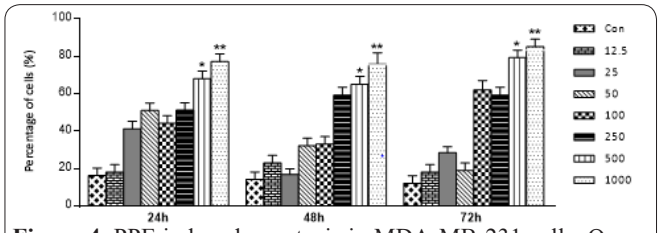

Figure 4. PPE induced apoptosis in MDA-MB-231 cells. Quantitative data indicate that total apoptosis significantly increased in treated cells with PPE (500 and 1000 µg/ml) after 24, 48 and 72h. * and ** donate P <0.05 and P <0.01, respectively, as compared to the control.

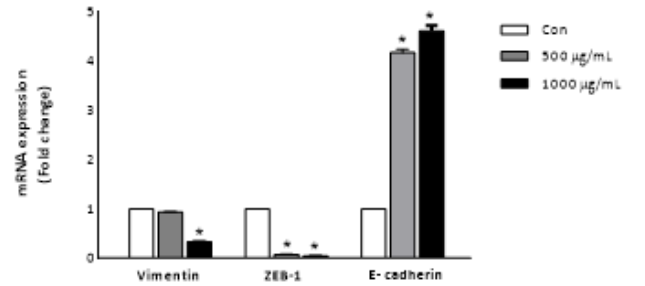

Figure 5. The effect of PPE on gene expression of EMT markers in TNBC cells. PPE altered the expression of vimentin, ZEB1 and E-cadherin. Relative levels are presented as fold change compared with untreated. Significance was set at *p<0.05.

induced apoptosis of MDA-MB-231 cells at maximum doses (500 and 1000 µg/ml); however, its antimigratory effect was exerted from the lower doses.

PPE decreased expression of genes involved in EMT

To better understand the effects of PPE on molecular factors involved in the metastatic process, the expression of specific genes involved in EMT including vimentin, ZEB1 and E-cadherin were examined. We found that PPE treatment strongly decreased the expression of EMT marker of vimentin at 1000 µg/ml PPE after 48h (Data not shown) and 72h (Figure 5). PPE also significantly decreased the expression of ZEB1 and increased E-cadherin expression at 500 and 1000 µg/ml PPE. These results indicate significant expression changes of EMT markers in TNBC cells following PPE treatment (500 and 1000 µg/ml).

β-catenin expression changed following PPE treatment in MDA-MB-231 cells

To further extend this study, the expression of β-catenin was evaluated. The result of our experiment showed that PPE treatment decreased the mRNA expression of β-catenin in MDA-MB-231 cells at 1000µg/ml PPE after 72h (Figure 6). PPE did not change the expression of β-catenin at lower doses and other time points (data not shown).

As shown in figure 7, employing immunoblot analysis, we found that β-catenin protein expression was decreased after treatment of cells with PPE at 1000 µg/ml for 24, 48 and 72h. Treatment with lower doses of PPE did not change β-catenin protein expression in MDA-MB-231 cells (data not shown).

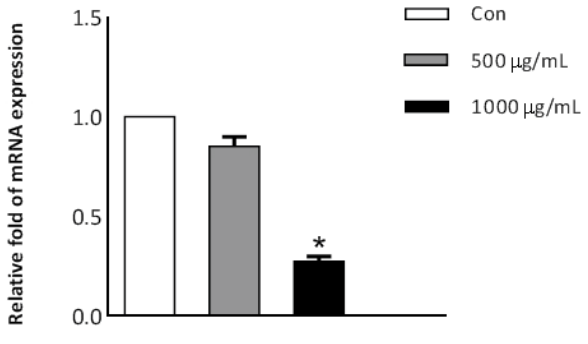

Figure 6. Fold changes in β-catenin mRNA expression following PPE treatment normalized to GAPDH mRNA. Total RNA was extracted from the cells treated with 500 and 1000ug/ml PPE after 72h. Significance was set at *p<0.05.

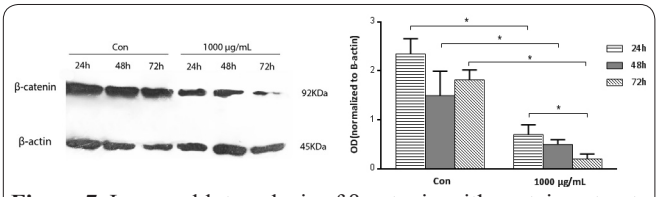

Figure 7. Immunoblot analysis of β-catenin with protein extracts from MDA-MB-231 cells treated with 1000 µg/ml PPE for 24, 48 and 72h compared with the control (untreated). (A) An original blot; (B) The result of densitometric analysis. * donates P <0.05.

Discussion

The metastatic nature of breast cancer remains the main cause of death in many patients who succumb to this disease (5). Unfortunately, current therapeutic strategies are not appropriate because of their high cost, many side effects and no guarantee against metastasis and cancer recurrence (3). In recent years, some studies have been reported the anticancer activity of pomegranate as a natural product (7). Different phytochemicals, including polyphenols, hydrolysable tannins (punicalagin, ellagic acid, gallic acid and gallagic acid), fatty acid (punicic acid), and anthocyanins (delphinidin, cyaniding and pelargonidin) were detected in pomegranate fruit (11). Compared to the whole fruit and pulp extracts, the peel extract showed the highest antioxidant activity (8, 9, 14). According to HPLC analysis, the most abundant phenolic acid detected in the PPE extract is ellagic acid followed by gallic acid, p-hydroxybenzoic acid, caffeic acid, and chlorogenic acid. These phenolic fractions are responsible for anticancer activity of PPE extract (15). The antiproliferative activity of methanolic extract of PPE was studied in breast, lung, ovarian and prostate cancer cell lines (16-18). Li et al. reported the antiapoptotic property of PPE via mitochondria-mediated apoptotic pathway (17). Deng et al. revealed that the extract from pomegranate peel induces apoptosis and inhibits metastasis in prostate cancer cells (18). Despite high antioxidant activity of peel part of the fruit, there are handful studies that aimed to evaluate its antimetastatic properties.

In this study, given the well-known limitations of metastatic breast cancer treatments, we explored the antimetastatic effects of PPE on TNBC cells in vitro and also studied the underlying possible mechanisms. Our results indicate that PPE markedly inhibited the migratory capabilities of the cells. Wang et al. showed that pomegranate juice retards cellular migration via downregulation of several genes involved in cell adhesion including ICAM-1, MARCKS, claudin 1 and also transcriptional factors involved in EMT (19, 20). Li et al. reported the antimigratory property of PPE via downregulation of MMP9 in thyroid cancer cell lines (17). Deng et al. showed that downregulation of MMP2/MMP9 and upregulation of tissue inhibitor of metalloproteinases 2 (TIMP2) are in accordance with the inhibition of migration and invasion in prostate cancer cells (18).

Over the last few years a wealth of reports has linked the overexpression of ZEB1 as an EMT marker to

increased metastasis in various types of human cancers (21, 22). Vice versa, knockdown of ZEB1 with siRNA in cancer cells such as H460 cells resulted in metastasis inhibition (23). Furthermore, vimentin plays a critical role in the EMT and its overexpression contributes to metastasis in numerous human cancers (24). Epithelial (E-) cadherin is an important adhesion molecule. Loss of E-cadherin leads to the breakdown of cell-cell adhesions and acquirement of invasive properties in cancer cells (25). Here, in an attempt to understand the underlying mechanism of PPE effect, the expressions of critical EMT markers of vimentin, ZEB1 and E-cadherin have been examined. A radical reduction of mRNA expression of ZEB1 and vimentin and also increased expression of E-cadherin have been observed in response to PPE treatment at maximum doses which indicate that the anti-migratory property of PPE might be mediated in part through its effect on EMT markers; however, this possible relationship needs to be verified by more experiments. Moreover, the results indicate that PPE does not change the expression of studied EMT markers at lower doses. Probably, the antimigratory effect of PPE at low doses is mediated through some other genes and proteins which should be examined in the future experiments.

Growing evidence suggests that Wnt/β-catenin signaling pathway plays a key role in the development and promotion of cancer metastasis (26). In the absence of Wnt signal, the cytoplasmic β-catenin is linked to a destruction complex (Axin, APC, CK1, GSK-3 beta), which facilitates β-catenin phosphorylation and degradation by proteasome. In the presence of a Wnt signal, the destruction complex is inactivated, which leads to stabilization and accumulation of β-catenin in the cytoplasm. Consequently, β-catenin translocates to the nucleus and binds to T-cell factor/lymphoid-enhancer factor, resulting in the activation of transcription of various target genes, such as CXCR4, CXCL12, MMP7, fibronectin, and hyaluronan synthase-2 (HAS2) which are involved in invasion and metastasis (27, 28). It was revealed that the Wnt pathway is more often activated in TNBC carcinomas than in other breast cancer subtypes (29). Matsuda et al. showed that Wnt/ β-catenin pathway activation stimulated tumor cell migration; conversely, ectopic expression of sFPR1 (Wnt inhibitor) reduced motility in MDA-MB-231 cells (30). Xu et al. reported that β-catenin knockdown strongly suppressed migration of MDA-MB-231 cells in comparison with control cells (31). In the present study, we tested the effect of PPE on the expression of β-catenin in NTBC cell line. A significant finding of this study is that decreased cell migration and increased apoptosis in MDA-MB-231 cells were associated with decreased expression of β-catenin. Consistent with our findings, Mandal et al. reported that pomegranate emulsion averts the expression, cytoplasmic accumulation, and nuclear translocation of β-catenin in mammary tumors in rat (11). Since down-modulation of β-catenin has been associated with inhibition of cell migration and induction of apoptosis, we assumed that suppression of Wnt/β-catenin signaling could be a possible mechanism of PPE-mediated inhibition of cell migration and escalation of apoptosis in TNBC cells. Previous data demonstrated that the inhibition of Wnt/β-catenin is essential for the chemosensitivity of breast cancer cells, and β-catenin can be an attractive therapeutic target for TNBC which lacks treatment targets due to its triple-negative nature (31). In view of these facts, PPE by decreasing the expression of β-catenin could be a potential candidate for cancer therapy especially in the case of TNBC. Further detailed work is necessary to fully elaborate the mechanism of antimetastatic and antiapoptotic effects of PPE on breast cancer cells. In conclusion, our data suggest that PPE could be added to the list of candidates for treatment of TNBC.

Acknowledgements
We thank Mr. Akhondi for technical support, preparing PPE. This study is funded by a grant from Shahroud university of Medical Sciences, Shahroud, Iran.

Conflicts of interest
The authors have no conflict of interest to declare.

References

1. Boyle P. Triple-negative breast cancer: epidemiological considerations and recommendations. Annals of oncology : official journal of the European Society for Medical Oncology. 2012;23 Suppl 6:vi7-12.
2. Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. Cancer biology & medicine. 2015;12(2):106-16.
3. Howard-Anderson J, Ganz PA, Bower JE, Stanton AL. Quality of life, fertility concerns, and behavioral health outcomes in younger breast cancer survivors: a systematic review. Journal of the National Cancer Institute. 2012;104(5):386-405.
4. Popolin CP, Reis JPB, Becceneri AB, Graminha AE, Almeida MAP, Correa RS, et al. Cytotoxicity and anti-tumor effects of new ruthenium complexes on triple negative breast cancer cells. 2017;12(9):e0183275.
5. Bertozzi S, Londero AP, Cedolini C, Uzzau A, Seriau L, Bernardi S, et al. Prevalence, risk factors, and prognosis of peritoneal metastasis from breast cancer. SpringerPlus. 2015;4:688.
6. Ahmadiankia N, Moghaddam HK, Mishan MA, Bahrami AR, Naderi-Meshkin H, Bidkhori HR, et al. Berberine suppresses migration of MCF-7 breast cancer cells through down-regulation of chemokine receptors. Iranian journal of basic medical sciences. 2016;19(2):125-31.
7. Panth N, Manandhar B. Anticancer Activity of Punica granatum (Pomegranate): A Review. 2017;31(4):568-78.
8. Li Y, Guo C, Yang J, Wei J, Xu J, Cheng S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food chemistry. 2006;96(2):254-60.
9. Modaeinama S, Abasi M, Abbasi MM, Jahanban-Esfahlan R. Anti Tumoral Properties of Punica Granatum (Pomegranate) Peel Extract on Different Human Cancer Cells. Asian Pacific journal of cancer prevention : APJCP. 2015;16(14):5697-701.
10. Elango S, Balwas R, Padma VV. Gallic acid isolated from pomegranate peel extract induces reactive oxygen species mediated apoptosis in A549 cell line. Journal of Cancer Therapy. 2011;2(05):638.
11. Mandal A, Bishayee A. Mechanism of Breast Cancer Preventive Action of Pomegranate: Disruption of Estrogen Receptor and Wnt/beta-Catenin Signaling Pathways. Molecules (Basel, Switzerland). 2015;20(12):22315-28.
12. Mishan MA, Ahmadiankia N, Matin MM, Heirani-Tabasi A,

Shahriyari M, Bidkhori HR, et al. Role of Berberine on molecular markers involved in migration of esophageal cancer cells. Cellular and molecular biology (Noisy-le-Grand, France). 2015;61(8):37-43.
13. Mishan MA, Heirani-Tabasi A, Mokhberian N, Hassanzade M, Kalalian Moghaddam H, Bahrami AR, et al. Analysis of Chemokine Receptor Gene Expression in Esophageal Cancer Cells Compared with Breast Cancer with Insights into Metastasis. Iranian journal of public health. 2015;44(10):1353-8.
14. Abdel Motaal A, Shaker S, editors. Anticancer and antioxidant activities of standardized whole fruit, pulp, and peel extracts of Egyptian pomegranate. The Open Conference Proceedings Journal; 2011.
15. Dikmen M, Ozturk N, Ozturk Y. The antioxidant potency of Punica granatum L. Fruit peel reduces cell proliferation and induces apoptosis on breast cancer. Journal of Medicinal Food. 2011;14(12):1638-46.
16. Modaeinama S, Abasi M, Abbasi MM, Jahanban-Esfahlan R. Anti tumoral properties of punica granatum (pomegranate) peel extract on different human cancer cells. Asian Pac J Cancer Prev. 2015;16:5697-56701.
17. Li Y, Ye T, Yang F, Hu M, Liang L, He H, et al. Punica granatum (pomegranate) peel extract exerts potent antitumor and anti-metastasis activity in thyroid cancer. RSC Advances. 2016;6(87):84523-35.
18. Deng Y, Li Y, Yang F, Zeng A, Yang S, Luo Y, et al. The extract from Punica granatum (pomegranate) peel induces apoptosis and impairs metastasis in prostate cancer cells. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2017;93:976-84.
19. Wang L, Alcon A, Yuan H, Ho J, Li QJ, Martins-Green M. Cellular and molecular mechanisms of pomegranate juice-induced anti-metastatic effect on prostate cancer cells. Integrative biology : quantitative biosciences from nano to macro. 2011;3(7):742-54.
20. Wang L, Ho J, Glackin C, Martins-Green M. Specific pomegranate juice components as potential inhibitors of prostate cancer metastasis. Translational oncology. 2012;5(5):344-55.
21. Zhang P, Sun Y, Ma L. ZEB1: at the crossroads of epithelial-mesenchymal transition, metastasis and therapy resistance. Cell cycle (Georgetown, Tex). 2015;14(4):481-7.
22. Chou YS, Yang MH. Epithelial-mesenchymal transition-related factors in solid tumor and hematological malignancy. Journal of the Chinese Medical Association : JCMA. 2015;78(8):438-45.
23. Fang S, Wu L, Li M, Yi H, Gao G, Sheng Z, et al. ZEB1 knockdown mediated using polypeptide cationic micelles inhibits metastasis and effects sensitization to a chemotherapeutic drug for cancer therapy. Nanoscale. 2014;6(17):10084-94.
24. Mendez MG, Kojima S, Goldman RD. Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2010;24(6):1838-51.
25. Peinado H, Portillo F, Cano A. Transcriptional regulation of cadherins during development and carcinogenesis. The International journal of developmental biology. 2004;48(5-6):365-75.
26. Li T, Lai Q, Wang S, Cai J, Xiao Z, Deng D, et al. MicroRNA-224 sustains Wnt/beta-catenin signaling and promotes aggressive phenotype of colorectal cancer. Journal of experimental & clinical cancer research : CR. 2016;35:21.
27. Kim JH, Park SY, Jun Y, Kim JY, Nam JS. Roles of Wnt Target Genes in the Journey of Cancer Stem Cells. International journal of molecular sciences. 2017;18(8).
28. Gao C, Xiao G, Hu J. Regulation of Wnt/beta-catenin signaling by posttranslational modifications. Cell & bioscience. 2014;4(1):13.
29. Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A, et al. beta-Catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2011;24(2):209-31.
30. Matsuda Y, Schlange T, Oakeley EJ, Boulay A, Hynes NE. WNT signaling enhances breast cancer cell motility and blockade of the WNT pathway by sFRP1 suppresses MDA-MB-231 xenograft growth. Breast cancer research : BCR. 2009;11(3):R32.
31. Xu J, Prosperi JR, Choudhury N, Olopade OI, Goss KH. beta-Catenin is required for the tumorigenic behavior of triple-negative breast cancer cells. PloS one. 2015;10(2):e0117097.