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ORIGINAL RESEARCH

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Anticancer potential of Marsdenia tenacissima extract: modulation of proliferation, apoptosis and *SBEM* gene expression in triple-negative breast cancer MDA-MB-231 cells

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Abstract

Marsdenia tenacissima (MT) is a herbal remedy that has been used for many years to treat cancer. While the anti-cancer properties of Marsdenia tenacissima extract (MTE) have been observed in several malignancies, its effect on the fundamental mechanisms of triple-negative breast cancer cells remains unclear. Here, we investigate the underlying anti-cancer mechanisms of MTE in MDA-MB-231 cells in regard to their proliferation, apoptosis, and the expression of the secretory breast epithelial mucin (*SBEM*) gene. Cell Counting Kit-8 (CCK-8) and Annexin V and propridium iodide (PI) staining kits were used to measure the proliferation and apoptosis in MDA-MB-231 cells, respectively. The effects of MTE on MDA-MB-231 cell migration were evaluated *via* scratch healing at 0 and 24 hours after treatment with different MTE concentrations. Western blot was used to detect the protein expressions of *SBEM* and apoptosis-related factors. Real-time quantitative Polymerase Chain Reaction (RT-qPCR) was performed to detect *SBEM* mRNA expression after different drug concentrations. CCK-8 assays indicated increased proliferation and inhibition of MDA-MB-231 cells with increasing MTE concentrations. Flow cytometry also showed a gradual dose-dependent increase in MDA-MB-231 cell apoptosis rate with increasing MTE concentrations. Additionally, MTE reduced the migration of MDA-MB-231 cells in a dose-dependent way. Proteomic experiments showed that the drug group downregulated the expression levels of B-cell Lymphoma-2 (BCL-2), B-cell lymphoma-extra large (BCL-XL) and *SBEM* in MDA-MB-231 cells, and RT-qPCR analysis showed that the expression of *SBEM* mRNA in MDA-MB-231 cells could be down-regulated in a dose-dependent manner by MTE. MTE exerted its anti-cancer effects on MDA-MB-231 cells by inhibiting their proliferation and migration, as well as inducing cell apoptosis. These effects might be associated with the down-regulation of BCL-2 and BCL-XL expression and the deregulation of *SBEM* mRNA expression.

Keywords

Marsdenia tenacissima extract; MDA-MB-231; *SBEM*; Proliferation; Apoptosis

1. Introduction

According to GLOBOCAN statistics, breast cancer is a significant global public health concern, with 2.3 million newly diagnosed cases, including male patients, and 685,000 deaths in 2020, and has now become the most commonly diagnosed cancer worldwide, surpassing lung cancer [1]. Despite the overall decline in cancer mortality rates by 33% since 1991 and the gradual decrease in the incidence of various cancer types, breast cancer incidence rates continue to rise, posing one of the major challenges in cancer prevention and treatment. Amongst women, breast cancer remains the leading cause of cancer-related deaths in developed countries, while developing countries experience the highest mortality rates, with significant racial disparities [2, 3]. Breast cancer comprises multiple molecular subtypes, among which triple-negative breast cancer (TNBC) represents a distinct subtype characterized by negative expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). TNBC accounts for approximately one-sixth of all breast cancer patients, and despite advances in the diagnosis and treatment of breast cancer, it is associated with poor clinical outcomes, possibly due to a lack of effective treatment targets [4–6]. Presently, it is considered that combination therapy with biomarkers is a fundamental strategy for improving the diagnostic rates and treatment outcomes of breast cancer patients, with current treatment options mainly

involving surgery, radiotherapy, and chemotherapy. Although chemotherapy remains the mainstay of treatment for TNBC, chemotherapy resistance may considerably reduce the patients' survival [7], which has been related to a lack of relevant therapeutic targets, leading to limited treatment efficacy [4, 8, 9]. Thus, identifying new effective biomarkers and therapeutic targets and selecting suitable biological therapeutic drugs is critical to improving the treatment efficacy of TNBC and prognosis.

MT, a perennial climber plant belonging to the Asclepiadaceae family, is widely distributed in the tropical and subtropical regions of Asia, particularly in southern China [10]. For thousands of years, MT has been used in Chinese traditional medicine [11]. Its medicinal properties were discovered and documented in the pharmacology book "Dian Nan Ben Cao" during the Ming Dynasty in China, approximately six centuries ago [12]. According to the Chinese Pharmacopoeia, MT has traditionally been used for the management of several human ailments, including asthma, tracheitis, rheumatism and carbuncle, among others [10, 13]. Furthermore, the root of MT has been reported to exhibit detoxifying, edema-reducing and analgesic effects [14]. Its traditional drug usage involves external application and decoction, while contemporary extraction technology has led to the development of intravenous medications such as Xiao-Ai-Ping injection. While its external application is mostly beneficial for localized swelling, discomfort and blisters, both decoctions and intravenous medications have been utilized to treat internal disorders, including malignancies. With the continuous advancement of modern pharmacological research and newer understanding of traditional Chinese medicine, numerous studies have been conducted on the medicinal properties of MTE, thereby revealing its therapeutic potential. MTE has demonstrated significant antitumor, hepatoprotective, diuretic, and immunomodulatory effects. Moreover, it has been shown to exert a synergistic effect on the suppression of tumor growth, indicating its potential use in cancer treatment [10, 15, 16]. MTE, also called Xiao-Ai-Ping injection, has been approved in China for cancer therapy [17], and existing studies have revealed its ability in inducing apoptosis [18], inhibiting angiogenesis, reversing drug resistance [12] and enhancing chemotherapy efficacy [15, 17, 19], as well as demonstrated its good auxiliary antitumor effect on lung cancer [20], liver cancer [21], esophageal cancer [11], breast cancer [22], among others.

Secretory breast epithelial mucin (*SBEM*) was initially discovered by Richard *et al*. [23] through isotopic labeling technology and gene expression analysis databases using public expression sequences. *SBEM* exhibits tissue-specific expression and is only expressed in breast and salivary gland tissues. Its expression level in primary breast tumors with axillary lymph node metastasis is reported to be significantly higher than in tumors negative for axillary lymph nodes, thereby suggesting its potential value in the clinical evaluation of breast cancer. Studies have identified the involvement of *SBEM* in tumor cell metastasis and possible association with the expression of epithelial-mesenchymal transition and calcium-binding proteins. Additionally, *SBEM*, as a biomarker, is highly expressed in breast cancer and was reported to be an independent risk prediction factor for evaluating the prognosis of breast cancer patients. Thus, this study aims to investigate the effects of MTE on TNBC cells and explore the mechanism of *SBEM* in MTE anti-tumor therapy.

Despite existing evidence demonstrating the anti-tumor effects of MTE, further exploration is warranted to comprehend its metabolic pathways and pharmacological mechanisms, as well as how it exerts its anti-tumor effects *in vivo*. The highly conserved programmed cell death mechanism, known as apoptosis, has been shown to be closely associated with tumorigenesis [24], tumor maintenance, and drug resistance [25–27]. Studies have shown that MTE can exert anti-cancer effects by inducing cell cycle arrest and apoptosis [28]. While MTE has shown inhibitory effects on various cancer types, its impact on TNBC remains poorly understood. Given the importance of apoptosis in triggering programmed cell death, we analyzed the anti-cancer activity of MTE in MDA-MB-231 cells through apoptotic signaling pathway analysis. Additionally, we explored the involvement of *SBEM* and apoptotic proteins in the anti-cancer mechanism of MTE.

2. Materials and methods

2.1 Cell cultures and reagents

To cultivate the human breast cancer cell line MDA-MB-231 (ATCC, Manassas, Virginia, USA), a complete culture medium containing 10% fetal bovine serum and 1% penicillin-streptomycin solution was prepared by mixing Dulbeo's modification of Eagle's medium Dulbeo (DMEM) (Thermo Fisher), fetal bovine serum, and penicillin-streptomycin solution. The cells were then incubated in the complete culture medium in a constant-temperature incubator. MTE (Trade name: Xiao-Ai-Ping injection, 202112091) (1 g crude/mL) was obtained from SanHome Pharmaceutical Co., Ltd (Nanjing, China).

The MTE concentration was expressed as the concentration of the original crude, as previously described [29]. The primary antibodies used were anti-β-actin (AF7018, 1:3000), anti-*SBEM* (NBP1-92366, 1:1000), anti-BCL-2 (AF6139, 1:1000) and anti-BCL-XL (AF6414, 1:1000), and the secondary antibody used was Horseradish Peroxidase (HRP) goat anti-rabbit Immunoglobulin G (IgG) (A0208, 1:1000), all of which were purchased from Affinity Biosciences (Jiangsu, China). The CCK-8 (121506, MCE, Shanghai, China) and RT-qPCR kits (67753, MCE, Shanghai, China) were purchased from MCE and the Annexin V-FITC/PI apoptosis kit (20220306, 4A Biotech, Suzhou, Jiangsu, China) from 4A Biotech. Analytical grade solvents and other compounds were also used in this experiment.

2.2 Proliferation assay

MDA-MB-231 cells in the logarithmic growth phase were evenly seeded at a density of 1×10^4 per well in a 96-well plate and incubated for 24 hours in an incubator kept at a constant temperature. After the cells adhered to the wall, the medium was replaced with a complete culture medium containing different concentrations of MTE. Five replicate wells were set up for each drug concentration, and the cells were further incubated under standard conditions for 24 hours, following which the original culture medium in the experimen-

tal wells was removed and replaced with a complete culture medium containing 10% CCK-8 reagent, which was then premixed and incubated for 2 hours in an incubator kept at a constant temperature. The absorbance value (optical density value, OD value) was measured at a wavelength of 450 nm using a microplate reader (UV-VIS spectrophotometer, Bio-Rad, Hercules, CA, USA), and cell viability was calculated. The experiments were conducted at least three times.

2.3 Apoptosis assay

MDA-MB-231 cells in the logarithmic growth phase were evenly seeded at a density of 1×10^5 per well in a 6-well plate and incubated in an incubator kept at a constant temperature. After 24 hours, the medium was replaced with a complete medium containing different MTE concentrations (0, 10, 20, 40, 60 and 80 mg/mL) and continued to culture for 24 hours, following which the culture medium was discarded, the cells were washed twice with phosphate buffered saline (PBS), digested with trypsin without ethylenediaminetetraacetic acid (EDTA), to which an appropriate amount of medium was added to terminate digestion. Then, a pipette tip was used to blow the cells to form a cell suspension, which was then collected into a centrifuge tube and centrifuged at about 1000 rpm for 5 minutes, following which the supernatant was carefully removed. Next, about 1 mL of pre-cooled PBS was added, and the cells were resuspended and centrifuged using the same method described above. Then, the supernatant was carefully removed and set aside. The cells were resuspended in $1\times$ binding buffer, the concentration was adjusted to 1×10^6 cells/mL, 100 μL of cell suspension was transferred to a 5 ml flow tube, 5 μL of Annexin V/FITC was added, the mixture was thoroughly mixed, and incubated for 5 minutes at room temperature in the dark. A flow cytometer (CYTEK, NL-3000, Shanghai Difa Instrument Co., Ltd, Shanghai, China) was used to detect the apoptosis rate of the cells.

2.4 Wound healing assay

To investigate the effect of MTE on the migration of MDA-MB-231 cells, the cells were adjusted to a density of 5×10^5 cells/mL and evenly seeded into a 6-well plate for 24 hours of culture under good growth conditions. Next, a scratch of around 0.6 mm width was created in each Petri dish using a 200 μL pipette tip, the cells in the culture dish were gently washed with PBS three times to remove any scraped cell debris and residual medium and incubated with a 2% low serum medium supplemented with solutions of different MTE concentrations (0, 10, 20, 40, 60 and 80 mg/mL). Then, the cells were visualized to determine how they migrate into the wounded area or protrude from the border of the wound using an inverted microscope at 0 hours and 24 hours in the same field of view. Data analysis was performed using the ImageJ software (version ImageJ.JS, National Institutes of Health, Bethesda, MD, USA).

2.5 Real-time quantitative RT-PCR (RT-qPCR)

To investigate the effect of MTE on the expression of *SBEM* mRNA in MDA-MB-231 cells, the cells were treated with different concentrations of MTE and incubated in a 37 °C constant temperature incubator. After 24 hours, the cells were removed from the incubator, the culture medium was discarded and washed three times with PBS, following which an appropriate amount of lysis buffer was added, and the mixture was transferred to a centrifuge tube for further use. Total RNA was then extracted from the lysed cells with RNAsimple (Tiangen Biochemical Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. cDNA synthesis was performed using extracted RNA, then qPCR was performed in triplicate by real-time PCR. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as the internal reference, and the primers for this experiments are shown in Table 1.

TABLE 1. PCR primers.

Gene	Primer sequence	Length of products (bp)
SBEM	5′-TCTGCCCAGAATCCGACAAC-3′ 5′-AATGTCTTTACGAGCAGTGGTAG-3′	168
GAPDH	5′-ATCCCATCACCATCTTCCAG-3′ 5′-GAGTCCTTCCACGATACCAA-3′	308

SBEM: small breast epithelial mucin; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

2.6 Western blot analysis

Different concentrations of MTE solution were added to MDA-MB-231 cell culture dishes and cultured in a constant temperature incubator for 24 hours. The cells were then dissolved in radio immunoprecipitation assay (RIPA) buffer (culture medium) (103393, Beyotime, Shanghai, China) to obtain protein extracts. The BCA protein assay kit (052319190729, Beyotime, Shanghai, China) was used to prepare a suitable volume of working solution by mixing A and B solutions in a ratio of 50:1, and the total protein content was measured following the manufacturer's instructions. Then, a 15% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) was prepared, and the proteins were separated by electrophoresis. The protein bands from the gel were then transferred onto a polyvinylidene fluoride (PVDF) membrane, which was treated with a blocking solution containing 5% nonfat milk for 1 hour at room temperature, incubated with a diluted primary antibody on a shaker overnight at 4 °C, to which a secondary antibody diluted with tris buffered saline with tween 20 (TBST) was added and incubated at room temperature for 1 hour. Enhanced chemiluminescence (ECL) detection reagents (P0018FS, Beyotime, Shanghai, China) were used to identify protein band signals.

2.7 Statistical analysis

Each experiment was performed at least three times, and the data were statistically evaluated using the GraphPad Prism software (version 9.4.1, GraphPad Software, San Diego, CA, USA). Significant differences between comparisons were con-

sidered for p values < 0.05. One-way analysis of variance (ANOVA) was used to analyze the differences between each experimental group, with the significance of differences between groups indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$.

3. Results

3.1 MTE inhibits the proliferation of breast cancer cell

To determine the cytotoxicity of MTE, the cells were treated with increasing MTE concentrations (10, 20, 40, 60 and 80 mg/mL), and CCK-8 assays were performed to determine the OD value of the MDA-MB-231 cells to ascertain the proliferation inhibitory effects of MTE. The results showed that MTE inhibited cell proliferation in MDA-MB-231 cell line in a concentration-dependent manner (Fig. 1).

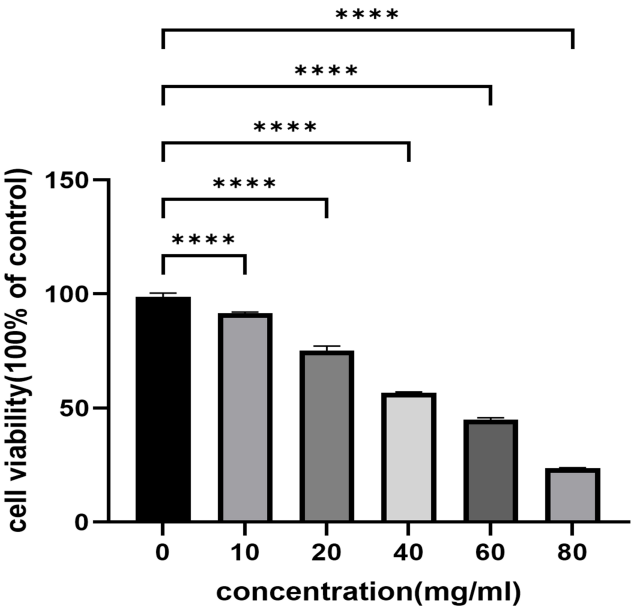

FIGURE 1. The effects of MTE on cell the inhibition of cell proliferation *via* CCK8 assay. MTE exhibited a concentration-dependent inhibitory effect in MDA-MB-231 cells, and the IC50 was 45 mg/mL. $****p < 0.0001$.

3.2 MTE promoted the apoptosis in MDA-MB-231 cells

To understand the relationship between the degree of growth inhibition of MDA-MB-231 cells and different concentrations of MTE, we investigated the apoptosis rates of MDA-MB-231 cells under the influence of different drug concentrations. The results of flow cytometry indicated that MTE effectively induced 4.08%, 6.37%, 12.5%, 22.4%, 34.3% and 43.0% cell apoptosis when MDA-MB-231 cells were subjected to 0 mg/mL, 10 mg/mL, 20 mg/mL, 40 mg/mL, 60 mg/mL and 80 mg/mL MTE for 24 hours, respectively (Fig. 2A). Fig. 2B displays the histograms of the MDA-MB-231 cell apoptosis rate. The results demonstrated that MTE triggered apoptosis in MDA-MB-231 cells in a dose-dependent manner.

3.3 Effects of MTE on MDA-MB-231 cell migration

To determine the effects of MTE on the migration of the cells, MDA-MB-231 cells in an excellent growth state were placed into 6-well plates and cultivated for 24 hours. Then, a 200-μL pipette tip was used to create wounds, and the cells were washed with PBS and incubated in 2% serum DMEM supplemented with MTE for 24 hours. An inverted microscope (Olympus) was used to monitor cell migration at 0 hour and 24 hours after scratching. Fig. 3A showed the migration of MDA-MB-231 cells after treatment with MTE. It was observed that the scratch almost completely closed at 0 mg/mL drug concentration, while MTE treatment (20 mg/mL, 40 mg/mL and 80 mg/mL) resulted in decreased cell migration in a dose-dependent manner. The migration images were processed using ImageJ to calculate the migration rate, and statistically significant differences were observed between the adjacent groups ($p < 0.01$) (Fig. 3B).

3.4 MTE suppresses SBEM expression in MDA-MB-231 cells

Above, we confirmed the expression of *SBEM* in breast cancer *via* multiple experiments. To understand the effects of MTE on *SBEM* expression in TNBC cells, we analyzed the expression levels of *SBEM* mRNA in MDA-MB-231 cells treated with different doses of MTE. The results are showed in Fig. 4, that MTE regulated the expression of *SBEM* mRNA in MDA-MB-231 cells, which led to increased inhibition following increased drug concentration.

3.5 MTE regulated the expression of apoptosis-related factors in MDA-MB-231 cells

The protein expression levels of relevant factors in MDA-MB-231 cells treated with different drug concentrations are shown in Fig. 5. Compared with the control group, the expression of anti-apoptosis regulators (*i.e.*, BCL-XL and BCL-2) and breast cancer-specific protein *SBEM* were found to decrease in the treated cells, indicating that as the concentration of MTE increases (10, 20, 40, 60, 80 mg/mL), the protein expression levels of BCL-2, BCL-XL and *SBEM* gradually decrease in MDA-MB-231 cells. Thus, the expression of the anti-apoptotic proteins and *SBEM* were downregulated in a concentration-dependent manner.

4. Discussion

The treatment of TNBC is particularly challenging due to limited treatment options. With the continuous research of modern pharmacology in traditional Chinese medicine, MTE has been found to have excellent anti-tumor activity. MTE, derived from the traditional Chinese herb MT, has been used for years to manage various types of cancers [11, 30–32], and its various anti-cancer actions have been confirmed in clinical trials. *In vitro* experiments have shown that MTE can exert anti-tumor effects by modulating the expression of various cytokines within cells and inducing cell apoptosis

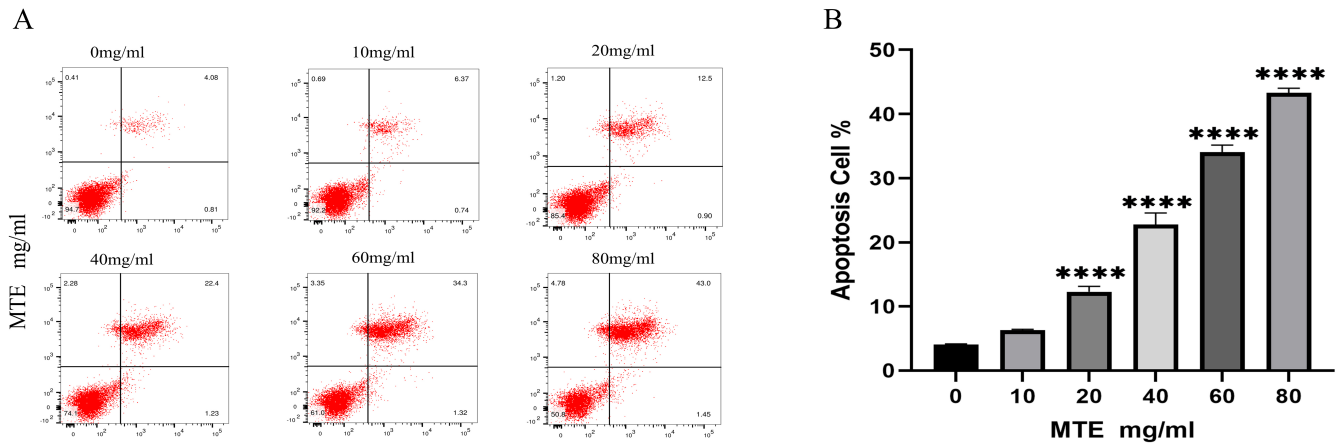

FIGURE 2. Effects of MTE on MDA-MB-231 cell apoptosis. MDA-MB-231 cells were plated into 6-well plates and treated with MTE (0, 10, 20, 40, 60 and 80 mg/mL) for 24 hours. Cell apoptosis was detected by Annexin V/PI staining and flow cytometry. MTE: marsdenia tenacissima extract; ****$p < 0.0001$.

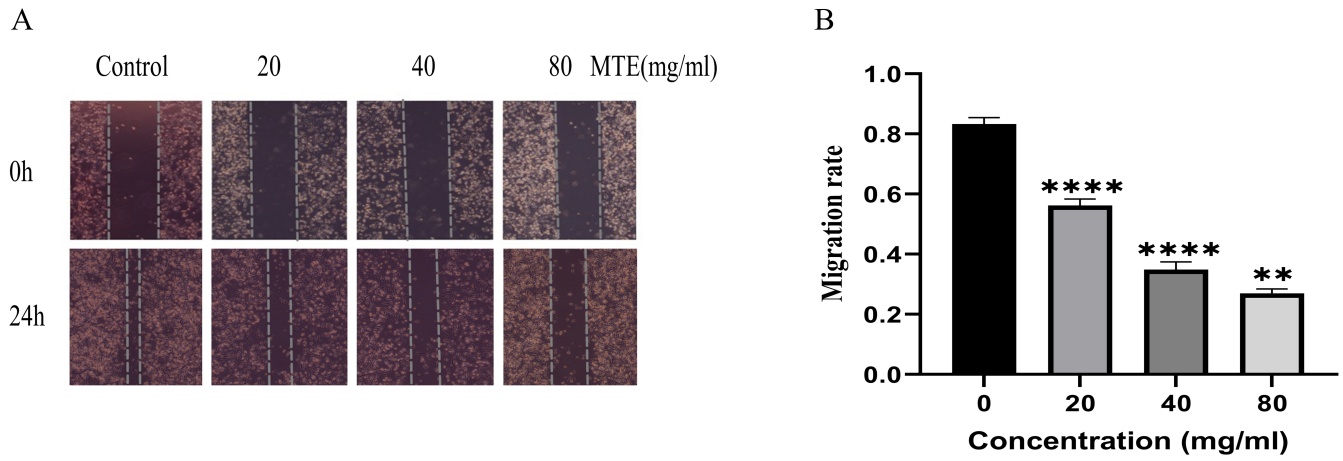

FIGURE 3. Effects of MTE on MDA-MB-231 cell migration. Images of the wound healing assay were taken at 24 h after scratching, and the MDA-MB-231 cells migration rate after 24 h. MTE: marsdenia tenacissima extract; **$p < 0.01$; ****$p < 0.0001$.

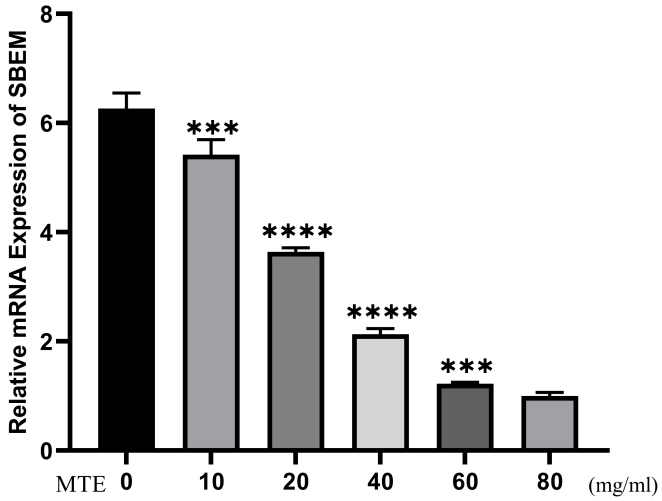

FIGURE 4. Expression of *SBEM* mRNA in MDA-MB-231 cells treated with different concentrations of MTE. MTE: marsdenia tenacissima extract; ***$p < 0.001$; ****$p < 0.0001$.

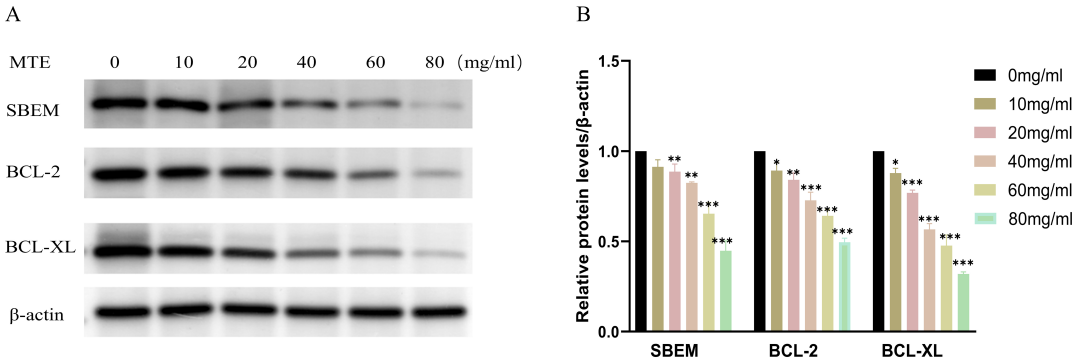

FIGURE 5. MDA-MB-231 cells were treated with increasing concentrations of MTE for 24 h, and the expression of *SBEM*, BCL-2 and BCL-XL were analyzed by western blot, with β-Actin used as the loading control. MTE: marsdenia tenacissima extract; *SBEM*: small breast epithelial mucin; BCL-2: B-cell Lymphoma-2; BCL-XL: B-cell lymphoma-extra large.

[10]. This present study revealed that MTE could impede the metastasis of different types of tumor cells while also triggering apoptosis. This effect was achieved by suppressing anti-apoptotic protein expression, namely BCL-2 and BCL-XL. Although MTE has shown tremendous clinical potential in the treatment of various tumors, its molecular mechanism in inhibiting tumor development has not been fully elucidated, and there is little research on its clinical efficacy in TNBC, thereby hindering its widespread clinical application.

SBEM exclusively exists in the mammary and salivary glands [23]. The results of experimental studies indicate that *SBEM* has the potential to serve as an independent risk predictor and could be a useful prognostic marker for TNBC patients. Researchers have found that changes in *SBEM* detected in patient tissues could be used as an indicator to effectively evaluate the blood-borne micro-metastasis of tumor cells in patients, with an effectiveness rate equivalent to that of pathological histology. In neoadjuvant chemotherapy for breast cancer, it was discovered that changes in the expression levels of *SBEM* before and after chemotherapy could effectively reflect the effectiveness of tumor chemotherapy, suggesting that *SBEM* might serve as an effective biomarker for evaluating micro-metastasis and prognosis of breast cancer patients [33, 34]. Poor patient prognosis and *SBEM* expression levels are positively correlated. The distant metastasis of tumor cells is an important component of tumor development, and the epithelial-mesenchymal transition (EMT) has been demonstrated to be a facilitator promoting a series of metastatic processes [35]. In this study, we showed that MTE could effectively inhibit the migration of MDA-MB-231 cells, which was concordant with previous studies that reported a role for *SBEM* in promoting the distant metastasis of tumor cells [36]. Specifically, we demonstrated that MTE down-regulated the expression of *SBEM*, which is likely one of the mechanisms *via* which MTE exerts its anti-metastatic effects. As previously stated, *SBEM* is overexpressed in breast cancer tumor cells, thereby inhibiting apoptosis and promoting tumor spread, which was confirmed in our present study. Following treatment with MTE, we observed a decrease in the proliferation of MDA-MB-231 cells and an increase in apoptosis. Additionally, we found that the expression of *SBEM* protein and gene was significantly reduced, indicating the potential role of *SBEM* in regulating the proliferation and apoptosis in breast cancer cells.

Apoptosis is a biologically regulated process of programmed cell death characterized by a series of biochemical and morphological changes that ultimately lead to the orderly disintegration and removal of dead cells, a typical characteristic linked to the development and maintenance of tumors, as well as resistance to chemotherapy. This might be because cancer cells often acquire the ability to evade apoptosis, allowing them to proliferate and survive despite cytotoxic treatments [37]. The BCL-2 protein family, which comprises pro-survival and pro-apoptotic subgroups, regulates intrinsic apoptosis [38]. Among them, the anti-apoptosis regulators include BCL-2 and BCL-XL, which make cells avoid apoptosis through the interaction of pro-apoptotic molecules [24, 39]. The current experiment demonstrated that MTE reduces the expression levels of BCL-2 and BCL-XL proteins, inhibiting proliferation and inducing apoptosis in MDA-MB-231 cells.

TNBC is a special subtype of breast cancer that is highly aggressive and has a poor prognosis. Due to its limited treatment options, mainly relying on chemotherapy, it has been a challenge in clinical practice. Breakthroughs in cancer treatment in recent years and the proposal of multiple treatment options, such as immune checkpoint inhibitors, PARP inhibitors, anti-angiogenic agents and gene-targeted therapy, have brought hope for improving the diagnostic efficacy and treatment outcomes of TNBC [40]. Unfortunately, effective immune checkpoints or gene targets have not yet been identified in TNBC, and no effective biomarkers are available for treatment decision evaluation. *SBEM* is a specific protein expressed in breast tissues and highly expressed in TNBC. This study found that it participates in regulating tumor cell growth and apoptosis and inhibits cell migration. Additionally, previous research has shown that *SBEM* can be an independent risk factor for TNBC and can be used to evaluate the efficacy of chemotherapy and participate in the EMT of tumor cells. These findings suggest *SBEM* as a promising biomarker for TNBC cells.

5. Conclusions

In conclusion, this study demonstrated that MTE exerts inhibitory effects on the proliferation of MDA-MB-231 cells

and promotes their apoptosis by regulating the release of anti-apoptotic regulatory factors. MTE also reduces the expression levels of *SBEM* mRNA in MDA-MB-231 cells. Therefore, MTE could potentially be considered a promising therapeutic candidate for the treatment of TNBC. Additionally, *SBEM* may serve as a specific tumor marker and a novel therapeutic target for breast cancer.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

LL and QX—created the study, offered the idea. QX and QYC—performed the study. QX, MC and MZ—wrote the article and took part in the data analysis. The final manuscript was reviewed and approved by all writers.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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