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Original Article

Piezo1-mediated cellular apoptosis in breast cancer cells triggered by ultrasound and microbubbles

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Abstract

Piezo1 ion channels play a crucial role in apoptosis regulation in human breast cancer cells (MCF-7), and this study evaluates the effects of Piezo1 agonist (Yoda1), inhibitor (GsMTx4), and ultrasound microbubble (USMB) treatment on cellular apoptosis pathways. In this research, in vitro cultures of normal breast epithelial cells (MCF-10A) and cancer cell lines (MCF-7, MDA-MB-231) were analyzed by Western blotting to determine Piezo1 protein levels, with MCF-7 selected for further analysis. Groups included control (untreated), Yoda1, USMB, GsMTx4, and USMB+GsMTx4, and apoptosis rates were measured via flow cytometry. Levels of apoptosis-related proteins (Bcl-2, Bax), endoplasmic reticulum stress proteins (GRP-78, Caspase 12), and mitochondrial pathway proteins (Cyt-c, Caspase 3, Caspase 9) were quantified, while JC-1 and Ca2+ fluorescent probes were used to assess mitochondrial membrane potential and intracellular Ca2+ concentration. Results showed MCF-7 cells expressed the highest Piezo1 levels. Yoda1 and USMB both markedly increased apoptosis, enhanced ER stress, and induced the mitochondrial apoptosis pathway in comparison to control, while GsMTx4 had the opposite effect and USMB reversed GsMTx4's phenotype. The USMB group exhibited the lowest mitochondrial membrane potential and the highest Ca2+ fluorescence intensity. These findings indicate that USMB activates ER stress via Piezo1, induces mitochondrial dysfunction, elevates intracellular Ca2+, and thereby promotes apoptosis in breast cancer cells.

Keywords: Ultrasound combined microbubbles, breast cancer, Piezo1, apoptosis, Ca^{2+}

1. Introduction

Breast cancer, being one of the most frequent malignancies, holds the highest incidence rate among women[1]. Even with various treatment modalities like surgery, gene/drug targeting, nanotechnology, and phototherapy approaches, patient survival rates remain limited[2]. Therefore, it is crucial to deeply investigate the molecular mechanisms underlying breast cancer progression, explore novel and more effective treatment methods, and identify specific molecular targets to improve early diagnosis and treatment, ultimately enhancing patient outcomes.

The widespread application of ultrasound technology has led to a deeper understanding of its biological effects. Ultrasound's application in biomedicine includes imaging, gene/drug delivery, nanotechnology, and computer science, playing a crucial role in disease diagnosis and treatment. With the continuous development of ultrasound medicine, molecular biology, and microbubble contrast technology, the biological effects of ultrasound combined with microbubbles (USMB) have become a hotspot in clinical oncology treatment research, showing great potential for development. Previous studies have reported that USMB can promote apoptosis in prostate cancer cells, liver cancer cells, ovarian cancer cells, and pancreatic cancer cells without damaging normal tissues[3-5]. Drzał et al. [6] found that trans-ultrasound combined with phospholipid-coated oxygen microbubbles could effectively deliver oxygen to tumor tissues in vivo in mice, resulting in sensitization to radiotherapy. Ultrasound combined with functionalized microbubbles produces microbubble volume pulsation, which generates fluid microfluidics around the microbubbles, resulting in localized shear and mechanical stresses on the cells, thus amplifying the mechanical effects of low-intensity pulsed ultrasound [7].

The study of the interaction of mechanosensitive ion channels (MSCs) with ultrasound is currently receiving a lot of attention. MSCs are a novel class of ion channels capable of sensing and responding to mechanical force stimuli, including shear stress induced by ultrasound stimulation, which induces cation flow [8]. Piezo1 and Piezo2, components of MSCs, were first discovered in mammalian mesenchymal stem cells in 2010 and were initially thought

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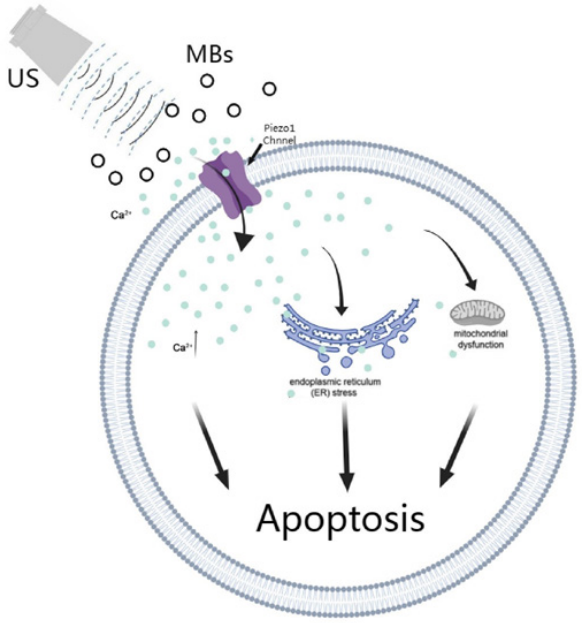

Fig. 1. Schematic diagram illustrating the promotion of apoptosis via Piezo1, a mechanosensitive ion channel protein, activated by USMB. The activation leads to Ca^{2+} influx, endoplasmic reticulum stress, and mitochondrial dysfunction, ultimately inducing apoptosis in MCF-7 cells.

to be related to embryonic development and shear force perception[9]. Previous studies have reported that Piezo1 is involved in the apoptosis of type II alveolar cells, degenerative nucleus pulposus (NP) tissue, prostate cancer, and pancreatic cancer[10-13]. However, there are no reports on whether USMB-induced apoptosis in breast cancer cells is related to Piezo1. This study aims to explore the role and possible mechanism of Piezo1 in the apoptosis of breast cancer MCF-7 cells induced by USMB from a molecular mechanism perspective, thereby validating our previous hypotheses (Fig. 1), providing experimental scientific evidence for new targeted therapies for breast cancer.

2. Materials and Methods

2.1. Cell culture and screening of the optimal cell line

Human normal breast epithelial cell line MCF-10A and breast cancer cell lines MCF-7 and MDA-MB-231 were purchased from the Cell Bank of the Chinese Academy of Sciences, Shanghai Institute of Cell and Biological Sciences. Human normal breast epithelial cells (MCF-10A) were cultured using a specialized medium for MCF-10A cells. Breast cancer cell lines MCF-7 and MDA-MB-231 were cultured in DMEM medium containing 1% penicillin/streptomycin and 10% fetal bovine serum. Cells were maintained in a 5% CO_2, 37°C cell incubator. Western blot analysis identified the Piezo1 protein expression levels across these cell lines, with the highest expressing line (MCF-7) chosen for further analysis.

2.2. Ultrasound combined with microbubble treatment

The therapeutic ultrasound apparatus WED-101 (Welld Medical Electronics Co., Ltd, China), diameter of its planar transducer of 45mm, a working frequency of 1MHz, a duty cycle 60% and a pulse repetition frequency of 100Hz, was used in this study. Preparation of Sono Vue (H20171213, Bracco, Italy) microbubble suspension: 5 ml of 0.9% saline for injection was added to Sono Vue lyophilized powder, shaken thoroughly until completely dissolved to form a sulfur hexafluoride microbubble suspension.

According to our team's preliminary screening results of the optimal USMB effect across various breast cancer cell lines, the ideal parameters for USMB irradiation in MCF-7 cells are as follows: a microbubble concentration of 30%, an ultrasound intensity of 0.5 W/cm², and an irradiation duration of 30 seconds[14]. Ultrasound combined with microbubble irradiation of cells: MCF-7 cells were evenly plated in the Petri dish. The ultrasound transducer was placed on the bottom of the Petri dish with the probe facing up, and coupler was applied to maintain a distance of 1cm between the transducer and the Petri dish (Fig. 2a).

2.3. Experimental grouping

This experiment was divided into Control, Yoda (M9372, AbMole), (Piezo 1 agonist added as a positive control group), USMB (ultrasound combined with microbubble irradiation group), GsMTx4 (M10039, AbMole) (Piezo 1 inhibitor added as an inhibitor control group), and USMB+ GsMTx4 group (ultrasound combined with microbubble irradiation + Piezo 1 inhibitor into inhibitor experimental group).

Cell drug intervention: The Piezo1 agonist Yoda1 was used at a concentration of 10 μmol/L for 2 hours. The Piezo1 inhibitor GsMTx4 was used at a concentration of 0.2 μmol/L for 1 hour. Proceed to the next phase of the experiment following the completion of the aforementioned intervention.

2.4. Flow Cytometry

Flow cytometry was used to detect cell apoptosis rates. Cells were stained with the Annexin V-FITC/PI apoptosis detection kit (400-6111-883, Yesen Biotech). After washing the cells twice with PBS, they were digested with EDTA-free trypsin and collected. The cells were then resuspended in 100 μL buffer, followed by the addition of 5 μL Annexin V-FITC and 10 μL PI. The cells were incubated at room temperature in the dark for 15 minutes, and apoptosis rates were detected using a flow cytometer (Beckman). Flow Jo software was used to analyze the apoptosis results.

2.5. Detection of Mitochondrial Membrane Potential

The JC-1 fluorescent probe kit (KGA603-1, KeyGEN BioTECH) was used to detect mitochondrial membrane

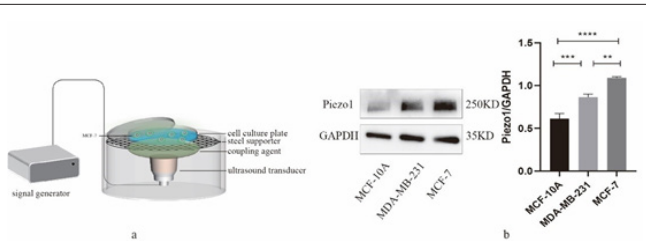

Fig. 2. Apparatus for cell irradiation in vitro and expression levels of Piezo1 protein in different cells. a. The ultrasonic transducer is positioned at the bottom of the culture dish, facing upward, with a coupler maintaining a 1 cm distance between the transducer and the dish. b. Compared to normal breast epithelial cells, Piezo1 expression levels were significantly elevated in the breast cancer cell lines MDA-MB-231 and MCF-7, with the highest increase observed in MCF-7 cells. $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

potential. After washing the confocal dish twice with PBS, 0.5 ml of pre-prepared JC-1 staining working solution was added and mixed thoroughly. The cells were incubated at 37°C in a 5% CO_2 incubator for 20 minutes. The working solution was discarded, and the cells were washed three times with 0.5 ml of JC-1 staining buffer. The cell slides were then mounted and preserved. Fluorescence intensity was detected and photographed under a fluorescence microscope (Zeiss) and analyzed using ImageJ.

2.6. Detection of Intracellular Ca^{2+} Concentration

The intracellular Ca^{2+} concentration was detected using Ca^{2+} fluorescent probes Fluo4-AM (KGAF024, KeyGEN BioTECH) and Rhod-2-AM (MX4506, Maokang Bio). The confocal dish was washed three times with Ca^{2+}-free Hank's balanced salt solution (HBSS). The Ca^{2+} fluorescent probes Fluo4-AM and Rhod-2-AM were diluted with HBSS to prepare the working solution. The fluorescent probe detection reagents were added according to the instructions. After incubating in the dark for 30 minutes, the intracellular Ca^{2+} concentration was detected using a fluorescence microscope and flow cytometer. The fluorescence intensity was analyzed using Flow Jo and ImageJ.

2.7. Western Blot

Western blot was used to detect the relative expression levels of apoptosis-related proteins (Bax, Bcl-2), endoplasmic reticulum stress-related proteins (GRP-78, Caspase12), and mitochondrial apoptosis pathway-related proteins (Cyt-c, Caspase 3, Caspase 9). RIPA cell lysis buffer with PMSF was prepared at a 100:1 ratio. Lysis buffer 1.0 mL was added to cells in different groups, lysed on ice for 30 minutes, and centrifuged at 12000 rpm for 15 minutes. The supernatant was collected, mixed with 4x protein loading buffer at a 4:1 ratio, and stored at -20°C. Proteins were separated on 12% SDS-PAGE gels at 120V, then transferred at a constant voltage of 100V for 90 minutes. The membranes were blocked for 1 hour and incubated with primary antibodies overnight at 4°C. The membranes were then incubated with secondary antibodies for 1 hour, followed by ECL detection. The gray values of the bands were quantified using ImageJ software. The primary antibodies used were: Piezo1 (AB259949, ABclonal), Bax (Q07812, Abways), Caspase-12 (A22864, Abways), Bcl-2 (A19693, ABclonal), Caspase-3 (A19654, ABclonal), and GRP78 (A23453, ABclonal).

2.8. Data Analysis

GraphPad Prism 8.0 was used for data processing and mapping. All experiments were independently repeated three times. The measurement data were represented by mean ± standard deviation ($\bar{x} \pm s$). *t-test* was used for comparison between two independent sample groups, *one-way* analysis of variance was used for comparison between multiple groups, and *Tukey-t test* was used for multiple comparisons between groups. The Dunnett-t test is employed to compare multiple experimental groups individually against a single control group. $P < 0.05$ was considered statistically significant.

3. Results

3.1. High Expression of Piezo1 in Breast Cancer Cell Line MCF-7

Western blot analysis showed that the relative content of Piezo1 protein in breast cancer cell lines was significantly higher than in human normal breast epithelial cells (MCF-10A). The expression level of Piezo1 in MDA-MB-231 was 1.7 times that of MCF-10A ($P < 0.05$), and in MCF-7, it was 2.1 times that of MCF-10A ($P < 0.05$) (Fig. 2b). Therefore, all subsequent experiments in this study were conducted using MCF-7 cell line.

3.2. USMB Activation of Piezo1 Channel Protein Promotes Apoptosis in MCF-7 Cells

Flow cytometry results revealed that the apoptosis rates of MCF-7 cells in the Control, Yoda1, USMB, GsMTx4, and USMB+GsMTx4 groups were 17.92±0.48%, 20.61±0.58%, 45.63±0.74%, 12.18±0.23%, and 25.98±0.36%, respectively (Fig 3a). Compared to the control group, the apoptosis rate in the Yoda1 group increased by 2.69% ($P < 0.05$) and by 27.71% in the USMB group ($P < 0.05$). In comparison to the USMB group, the apoptosis rate in the USMB+GsMTx4 group decreased by 19.65% ($P < 0.05$). Relative to the GsMTx4 group, the USMB+GsMTx4 group exhibited an increase in the apoptosis rate by 13.8% ($P < 0.05$).

Western blot results demonstrated that, compared to the Control group, the relative expression of Bax protein was upregulated and that of Bcl-2 protein was downregulated in both the Yoda1 and USMB groups, with statistically significant differences between the groups ($P < 0.05$). Compared to the USMB group, the relative expression of Bax protein was downregulated and that of Bcl-2 protein was upregulated in the USMB+GsMTx4 group, with statistically significant differences between the groups ($P < 0.05$). Compared to the GsMTx4 group, the relative expression of Bax protein was significantly upregulated and that of Bcl-2 protein was downregulated in the USMB+GsMTx4 group, with statistically significant differences between the groups ($P < 0.05$) (Fig. 3b). This indicates that cells in the USMB group exhibit a higher potential for apoptosis. Activation of Piezo1 and USMB can promote apoptosis in MCF-7 cells, whereas the use of a Piezo1 inhibitor can prevent apoptosis in MCF-7 cells.

3.3. USMB Initiates Endoplasmic Reticulum Stress System Through Activation of Piezo1

Western blot results showed that, compared to the

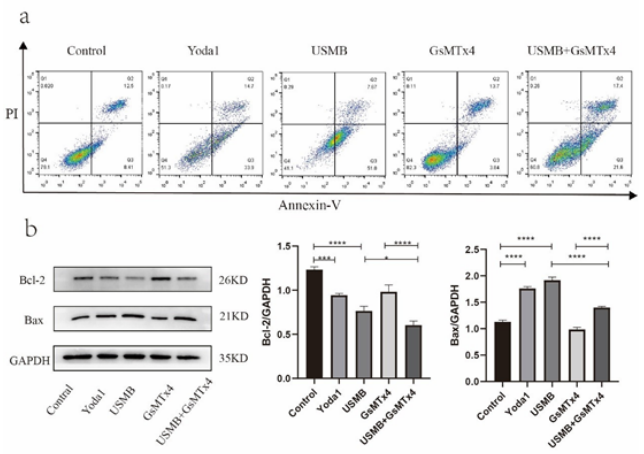

Fig. 3. Detection of apoptosis levels in different groups. a. Flow cytometry analysis of apoptosis rates in different groups. b. Western blot analysis of Bax and Bcl-2 protein expression levels in different groups. * P <0.05, **P<0.01, *** P <0.001, **** P <0.0001.

Control group, the relative expression levels of GRP78 and Caspase-12 proteins were upregulated in both the Yoda1 and USMB groups, with the highest expression observed in the USMB group. The differences between the groups were statistically significant ($P < 0.05$). Compared to the USMB group, the relative expression levels of GRP78 and Caspase-12 proteins were downregulated in the USMB+GsMTx4 group, with statistically significant differences between the groups ($P < 0.05$). Compared to the GsMTx4 group, the relative expression levels of GRP78 and Caspase-12 proteins were significantly upregulated in the USMB+GsMTx4 group, with statistically significant differences between the groups ($P< 0.05$) (Fig. 4). This indicates that USMB and Yoda1 can activate endoplasmic reticulum stress to promote cell apoptosis through activing Piezo1, while GsMTx4 can inhibit endoplasmic reticulum stress, thereby preventing cell apoptosis through inhibiting Piezo1.

3.4. USMB Induces Mitochondrial Dysfunction Through Activation of Piezo1

Western blot results showed that, compared to the Control group, the relative expression levels of Cyt-c, Caspase-3, and Caspase-9 proteins were upregulated in both the USMB and Yoda1 groups, with the highest expression observed in the USMB group. The differences between the groups were statistically significant ($P< 0.05$). Compared to the USMB group, the relative expression levels of Cyt-c, Caspase-3, and Caspase-9 proteins were downregulated in the USMB+GsMTx4 group, with statistically significant differences between the groups ($P< 0.05$). Compared to the GsMTx4 group, the relative expression levels of Cyt-c, Caspase-3, and Caspase-9 proteins were significantly upregulated in the USMB+GsMTx4 group, with statistically significant differences between the groups ($P < 0.05$) (Fig. 5a).

Fluorescent probe JC-1 was used to detect mitochondrial membrane potential. Results from fluorescence microscopy showed that in the control group, JC-1 predominantly existed in its aggregated form within cells, emitting an orange-red fluorescence, indicating a high mitochondrial membrane potential. In the USMB group and the Yoda1 group, JC-1 primarily appeared in its monomeric form, with weaker red fluorescence and stronger green fluorescence. Among them, the USMB group exhibited the weakest red fluorescence and the strongest green fluorescence, suggesting the lowest mitochondrial membrane potential and the most severe mitochondrial dysfunction. Compared to the USMB group, the GsMTx4+USMB group showed an increase in red fluorescence, indicating that the Piezo1 inhibitor alleviated the mitochondrial dysfunction induced by USMB (Fig. 5b). Based on these findings, it can be concluded that USMB-induced apoptosis is associated with mitochondrial dysfunction, and the activation of the Piezo1 channel contributes to this process by exacerbating mitochondrial impairment.

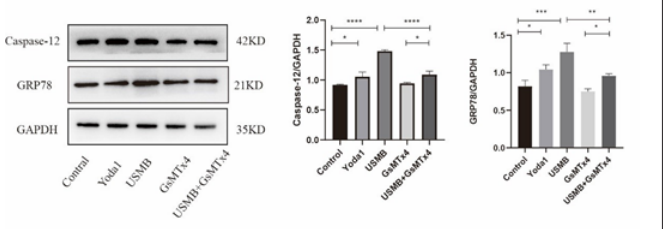

Fig. 4. Western blot analysis of the relative expression levels of endoplasmic reticulum stress-related proteins GRP78 and Caspase-12 in different groups. *P<0.05, ** P <0.01, *** P<0. 001, ****P<0.0001.

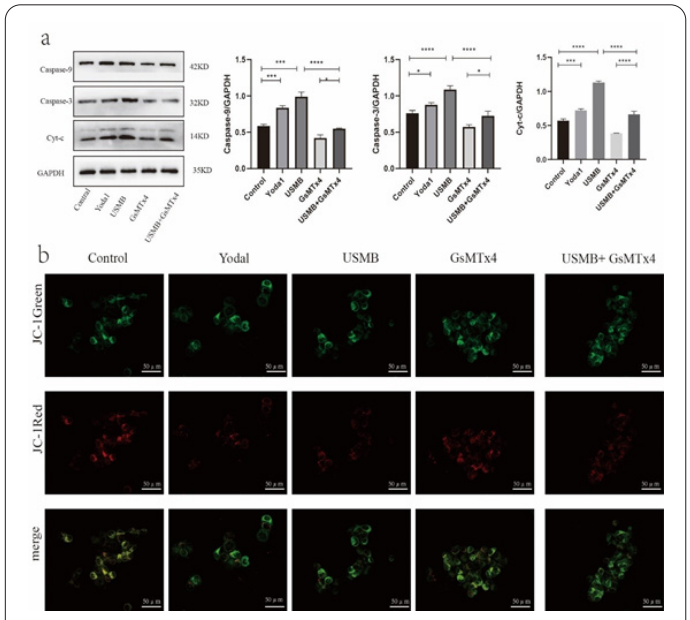

Fig. 5. Detection of mitochondrial dysfunction. a. Western blot analysis of the relative expression levels of mitochondrial apoptosis-related proteins Cyt-c, Caspase-3, and Caspase-9. *P<0.05, ***P<0.001, ****P<0.0001. b. Mitochondrial membrane potential was detected using the JC-1 probe. Scale bar: 50μm. When JC-1 exists in a polymeric form within the cells, it primarily emits red fluorescence. In its monomeric form, it predominantly emits green fluorescence.

3.5. Piezo1 is involved in partial Ca^{2+} influx in MCF-7 cells under the action of USMB

Using Ca^{2+} fluorescence probes to detect intracellular Ca^{2+} concentration, fluorescence microscopy results revealed that USMB treatment can induce intracellular Ca^{2+} influx (Fig 6a).

Compared to the Control group, intracellular Ca^{2+} concentrations were elevated in the Yoda1 and

USMB groups, with the highest fluorescence intensity observed in the USMB group. Compared to the USMB group, the intracellular Ca^{2+} fluorescence intensity in the USMB+GsMTx4 group was reduced. In contrast, compared to the GsMTx4 group, the intracellular Ca^{2+} fluorescence intensity in the USMB+GsMTx4 group was increased. We further quantified intracellular Ca^{2+} concentrations using flow cytometry (Fig. 6b), and the results were consistent with those from fluorescence microscopy. The intracellular Ca^{2+} concentrations from highest to lowest were as follows: USMB, Yoda1, USMB+GsMTx4, Control, and GsMTx4. Significant differences were observed between the USMB and USMB+GsMTx4 groups, as well as between the GsMTx4 and USMB+GsMTx4 groups (all $P< 0.05$). This indicates that part of the Ca^{2+} influx is mediated by the Piezo1 channel.

4. Discussion

Piezo1 is a mechanosensitive ion channel protein intricately linked to tumorigenesis. Previous research has predominantly explored the role of Piezo1 in cancers such as lung, gastric, and colorectal cancers. These studies have uncovered that Piezo1 exerts differential effects

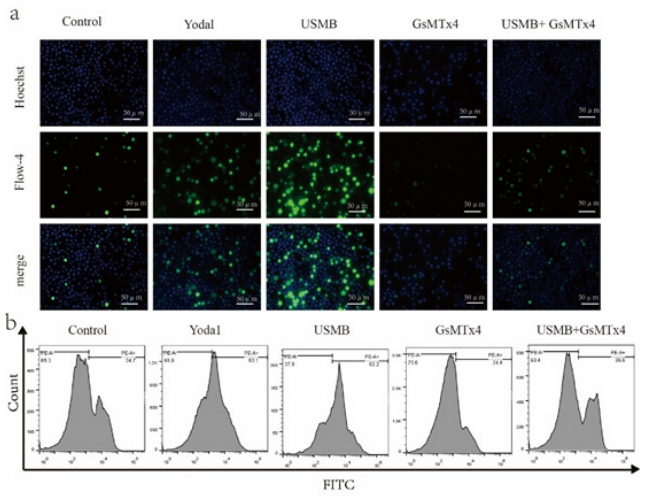

Fig. 6. Detection of intracellular Ca^{2+} levels. a. Fluo-4AM is a Ca^{2+} fluorescent probe. Hoechst staining was used for live cell nuclear fluorescence staining. Scale bar: 50μm. The fluorescence intensity of intracellular Ca^{2+} was visualized under a light microscope. b. Flow cytometry was used to measure intracellular Ca^{2+} concentrations, with results consistent with those observed by fluorescence microscopy.

on key cellular functions such as apoptosis, proliferation, and invasion, with its impact varying significantly across different types of tumors[15]. To date, the role of Piezo1 in breast cancer cell apoptosis remains unexplored. Therefore, this study explores the role and potential mechanisms of Piezo1 in the apoptosis of MCF-7 breast cancer cells induced by USMB.

This study demonstrates that USMB induces apoptosis in MCF-7 cells, while the addition of GsMTx4 effectively inhibits this apoptotic effect. These findings suggest that Piezo1 plays a crucial role in the USMB-mediated apoptosis of MCF-7 cells. The underlying mechanism may be that when ultrasound acts on cells, it generates physical force through the mechanical vibrations of sound waves, commonly referred to as shear force. This shear force exerts its influence on the mechanical receptors both on the cell membrane surface and within the cell, activating the ion channel protein Piezo1, which subsequently regulates the physiological functions and signaling pathways of the cell[8].

Endoplasmic reticulum (ER) stress is a vital regulator of apoptosis. The ER is a dynamic organelle involved in several essential functions such as calcium homeostasis, protein folding, lipid metabolism, and the initiation of apoptosis[16]. GRP78, a major ER stress marker, is associated with increased apoptosis when overexpressed[17]. ER stress also triggers the activation of the Caspase protein family, notably Caspase 12, which is linked to the ER membrane, though its specific role in apoptosis remains debated[18]. In this study, USMB was shown to enhance the expression of endoplasmic reticulum stress markers GRP78 and Caspase-12, thereby activating the endoplasmic reticulum stress-induced apoptosis pathway and promoting apoptosis in MCF-7 cells. In MCF-7 cells treated with GsMTx4, endoplasmic reticulum stress was suppressed following USMB treatment. Thus, Piezo1 plays a positive regulatory role in the USMB-induced endoplasmic reticulum stress pathway, facilitating the occurrence of apoptosis in MCF-7 cells.

Mitochondria, as the main ATP producers, are central to processes like apoptosis, aging, cancer progression, and cell signaling. Studies confirm that mitochondria are crucial organelles within the apoptotic regulatory network[19]. A key early event in apoptosis is the reduction in mitochondrial membrane potential, preceding nuclear changes like chromatin condensation and DNA fragmentation. A substantial drop in mitochondrial membrane potential marks the point of no return in apoptosis, underscoring mitochondria's critical role in this process. In this study, we assessed mitochondrial dysfunction using the Cyt-c marker and JC-1 fluorescent probe to track mitochondrial membrane potential alterations, Meanwhile, the expression levels of mitochondrial apoptosis-related proteins, Caspase-9 and Caspase-3, were also detected. In cells treated with USMB, the release of Cyt-c was highest, and the relative expression levels of Caspase-9 and Caspase-3 were significantly upregulated. The addition of GsMTx4 followed by USMB treatment, reduced the release of Cyt-c and the expression of Caspase-9 and Caspase-3. This further confirms that the mitochondrial dysfunction induced by USMB in MCF-7 cells is partially mediated by Piezo1.

Intracellular Ca^{2+} is the most abundant second messenger in the human body, and studies have shown that excessive Ca^{2+} can activate the endoplasmic reticulum stress pathway, thereby promoting apoptosis (20). Furthermore, high concentrations of Ca^{2+} can activate calcium-dependent enzymes, which play critical roles in various apoptotic signaling pathways, facilitating their interaction with the anti-apoptotic protein Bcl-2, leading to apoptosis (21). In this study, we found that apoptosis in MCF-7 cells increased as intracellular Ca^{2+} levels rose. The Ca^{2+} levels in MCF-7 cells of the USMB group were significantly higher than those in the control group, while the levels in the USMB+GsMTx4 group were significantly lower than those in the USMB group. We attribute this to the shear forces generated by USMB acting on the cell membrane, activating the Piezo1 ion channel, which promotes the influx of extracellular Ca^{2+} into the cell, subsequently inducing apoptosis. Therefore, during USMB-induced apoptosis, Ca^{2+} enters the cell through the Piezo1 ion channel, acting as a second messenger to activate endoplasmic reticulum stress, thereby promoting the release of apoptotic initiators.

In our study, we examined apoptosis, ER stress, mitochondrial dysfunction, and intracellular Ca^{2+} changes in MCF-7 cells under USMB exposure. The results suggest that USMB-generated mechanical forces activate Piezo1, leading to Ca^{2+} overload, which in turn activates both the ER stress and mitochondrial apoptosis pathways, culminating in breast cancer cell apoptosis. However, this study did not include the use of chemotherapeutic agents or targeted therapies. Previous research indicates that combining USMB with siRNA or chemotherapy can enhance treatment efficacy and reduce drug side effects[20]. Finally, given the expression of Piezo1 in breast cancer cells, we aim to establish an in situ MCF-7 model or a genetically engineered mouse model to further investigate the underlying mechanisms. This approach may provide valuable insights into mechanotransduction-based strategies for breast cancer therapy.

5. Conclusion

Piezo1 is involved in the USMB-induced apoptosis

of MCF-7 cells. The potential mechanism is that USMB triggers endoplasmic reticulum stress via the ion channel protein Piezo1, leading to mitochondrial dysfunction, increased intracellular Ca^{2+} concentrations, and consequently promoting apoptosis in MCF-7cells. These findings not only contribute to the understanding of novel therapeutic approaches but also provide a new avenue for the clinical management of breast cancer.

Abbreviations

USMB=ultrasound combined with microbubble; HBSS=Hank's balanced salt solution; ER=Endoplasmic reticulum

Author contributions statement

Nina Qu and Menglu Bi designed, conceived the study, and performed experiments. Qingkai Meng wrote the main manuscript text. Menglu Bi and Hexiu Liu prepared figures and tables. All authors participated in the data analysis and writing of the initial draft. Xiaoli Cao and Jingbo Wang were involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript. Nina Qu and Menglu Bi contributed equally to this work and should be regarded as co-first authors.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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