Archives of Biochemistry and Biophysics 646 (2018) 10–15

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Tubeimoside V sensitizes human triple negative breast cancer MDA-MB-231 cells to anoikis via regulating caveolin-1-related signaling pathways

Ke Wang[a], Xue Zhu[a], Yu Chen[b], Yongxiang Yin[c,*], Tao Ma[d,**]

[a] Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine, Wuxi 214063, Jiangsu Province, China
[b] Central Laboratory, The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University, Wuxi 214002, Jiangsu Province, China
[c] Department of Pathology, The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University, Wuxi 214002, Jiangsu Province, China
[d] Department of Breast, The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University, Wuxi 214002, Jiangsu Province, China

ARTICLE INFO

Keywords:
Triple-negative breast cancer
Anoikis resistance
Tubeimosides V
Caveolin-1

ABSTRACT

Metastatic triple-negative breast cancer (TNBC) has poor outcome with conventional chemotherapy regimens due to its aggressive behavior. The acquisition of anoikis resistance, a programmed cell death process triggered by substratum detachment, is an important mechanism in TNBC metastasis. Therefore, agents that can restore the sensitivity of cancer cells to anoikis may be helpful for the treatment of metastatic TNBC. In this study, we investigated the inhibitory effect of Tubeimosides V (TBMS-V), a cyclic bisdesmoside isolated from the ethanol extracts of tubers of B. paniculatum., on anoikis resistance and the involvement of caveolin-1(CAV-1)-related signaling pathways in such process in MDA-MB-231 cells. The results showed that the treatment of TBMS-V could sensitize cancer cells to anoikis, which was associated with suppression of anchorage-independent culture-induced CAV-1 overexpression, EGFR activation as well as ITGB1-FAK activation. The data from this study might contribute to providing a potential therapeutic target for metastatic TNBC and suggest the possibility of TBMS-V and its derivatives for metastatic TNBC therapy.

1. Introduction

Among women worldwide, breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer death [1,2]. Triple negative breast cancer (TNBC), defined by negative expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), accounts for approximately 15–25% of all breast cancer, and is more difficult to treat than other types of breast cancers due to the lack of therapeutic targets [3–5]. In these decades, survival rate of TNBC is significant improved by benefiting from the advances of early diagnosis, surgical techniques, and local and systemic adjuvant therapies [6,7]. However, the prognosis of patients with metastatic TNBC is still poor with a median survival of 13 months [8]. Thus, understanding the mechanisms responsible for TNBC metastasis would contribute to the development of new targeted therapeutic method for such disease.

Tumor metastasis is a multistep biological process including cancer cells detaching from the primary tumor by invasion, disseminating throughout the body by circulation, and colonizing at distant organs [9,10]. Anoikis (apoptosis resulting from loss of cell–matrix interactions) is suggested to be a barrier of metastasis; however, acquisition of anoikis resistance allows survival of cancer cells during systemic circulation, thereby facilitating secondary tumor formation in distant organs [11,12]. For this reason, anoikis induction may confer clinical benefit for metastatic cancers such as TNBC. Multiple mechanisms are involved in anoikis resistance but still not completed understood [13]. Caveolin-1 (CAV-1) belongs to caveolin family in caveolae membrane and plays an important role in several cancer-related processes, which include tumor growth, metastasis and angiogenesis [14–16]. The role of CAV-1 in breast cancer is an active area of investigation due to its dual role as tumor suppressor or as an oncogene [17,18]. The data from recent clinical and experimental studies have confirmed the important correlation of CAV-1 and high mortality rate in metastatic TNBC [19,20]. Existed evidence has estimated that CAV-1 contributes to tumor metastasis due to its ability of inducing anoikis resistance in cancer cells [21,22]. However, the role of such protein in acquirement of anoikis resistance in metastatic TNBC is still unclear.

Traditional Chinese Medicine (TCM) is applied for prevention and

* Corresponding author. Department of Pathology, The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University, Wuxi 214002, Jiangsu Province, China.
** Corresponding author. Department of Breast, The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University, Wuxi 214002, Jiangsu Province, China.E-mail:
E-mail addresses: yinyongxiang0118@outlook.com (Y. Yin), matao0202@outlook.com (T. Ma).

https://doi.org/10.1016/j.abb.2018.03.029
Received 2 February 2018; Received in revised form 15 March 2018; Accepted 22 March 2018
Available online 24 March 2018
0003-9861/

treatment of various diseases in Asian for many of years and now also the important resource for modern drug development [23–25]. Tu Bei Mu, the tuber of Bolbostemma paniculatum (Maxim.) Franquet (Cucurbitaceae), is a Chinese folk medicine with significant anti-tumor and anti-inflammatory effects. Tubeimosides V (TBMS-V) is a new minor constitute isolated from the ethanol extracts of tubers of B. paniculatum and estimated to has anti-tumor activity in human glioblastoma cells [26]. In this study, the inhibitory effect of TBMS-V on anoikis resistance and the involvement of CAV-1-related signaling pathways in such process were investigated in MDA-MB-231 cells, the *in vitro* model of human triple negative breast cancer.

2. Materials and methods

2.1. Reagents

Tubeimoside V (purity > 98%) was a kind gift from Institute of Medicinal Biotechnology (Beijing, China). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma (MO, USA). Annexin V-FITC and PI double staining kit was from BD Biosciences (CA, USA). The antibodies used in this study were obtained from Cell Signaling Technology (MA, UCA), Abcam (MA, UCA) and Santa Cruz Biotechnology (CA, USA). All other chemicals and reagents were purchased from Beyotime (Nantong, China).

2.2. Cell culture and transfection

The human triple negative breast cancer cell line MDA-MB-231 was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. MDA-MB-231 cells were cultured in Deulbecco's modified eagle medium (DMEM) medium supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. For the analysis of function of TBMS-V, cells were transfected with the indicated expression plasmids using lipofectamine2000 before treatment with agent.

2.3. Anoikis induction assay

Anoikis was induced by poly-HEMA culture as mentioned before [27]. Culture plates were coated with poly-HEMA (0.95 μL/mm^2, 50 mg/mL) and dried overnight at room temperature. Prior to use, cells were kept in suspension in DMEM medium supplemented with 10% FBS by using poly-HEMA coated culture plates to prevent adhesion.

2.4. Cell viability assay

Cell proliferation was measured by MTT assay as mentioned before [28]. After treatment, MTT (5 mg/mL) was added to each well and cells were incubated at 37 °C for 4 h. Then the culture medium was removed and 200 μL of DMSO was added to dissolve the formazan product. Cell viability was assessed by measurement with an ELISA reader (Bio-Rad, CA, USA).

2.5. Cell apoptosis assay

Cell apoptosis analysis was performed by double staining cells with the Annexin V-FITC and PI as mentioned before [29]. After treatment, cells were washed twice with ice-cold PBS, re-suspended in binding buffer and stained with Annexin V-FITC and PI at room temperature for 15 min in the dark. Cell apoptosis was assessed by flow cytometry (Becton-Dickinson, CA, USA).

2.6. Co-immunoprecipitation assay

After treatment, cell exacts were incubated with primary antibody at 4 °C overnight. Then protein A agarose beads were added and incubated with mild rocking at 4 °C for 3 h The immunoprecipitated proteins were eluted by heat treatment at 100 for 5 min with 2X sampling buffer. The input, immunoprecipitate and flow through fractions were analyzed by western blot analysis.

2.7. Western blot analysis

After treatment, protein sample was prepared from cells and separated by SDS-PAGE (10%) and electro-transferred onto polyvinylidene fluoride (PVDF) membrane. After blocked, the membrane was incubated with the primary antibody at 4 °C for overnight and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody at 37 °C for 2 h. The detection of protein bands was performed using the enhanced chemiluminescence (ECL) detection kit. The expression of β-actin was used as an internal control.

2.8. Statistical analysis

Biostatistical analyses were conducted with the GraphPad Prism 5.0 (GraphPad, CA, USA) and SPSS 16.0 software package (IL, USA). All experiments were done in triplicates and the results were indicative of three independent studies. Results of multiple experiments were expressed as means ± SD. A p value less than 0.05 was accepted as statistically significant.

3. Results

3.1. TBMS-V reverses anoikis resistance of MDA-MB-231 cells

Anoikis resistance is a hallmark of metastatic cancer such as triple negative breast cancer [30,31]. In this study, we investigated the potential effect of TBMS-V on anoikis resistance in MDA-MB-231 cells. First, morphological observation showed cells cultured in suspension formed multicellular spheroids, which promised the ability of cell proliferation in detached state; however, the treatment of TBMS-V decreased the number and size of spheroids (Fig. 1A). Then, MTT assay showed that the viability of cells in suspension was significantly reduced from 98.3% ± 3.17%–22.19% ± 2.07% after the treatment of TBMS-V (5 μM) for 24 h (Fig. 1B). Finally, flow cytometry analysis showed that the treatment of TBMS-V (5 μM) for 24 h significantly induced the apoptotic percentage of cells in suspension from 4.88% ± 2.96%–48.63% ± 4.98%, indicating the reduced cell viability was associated with apoptosis (Fig. 2).

3.2. TBMS-V sensitizes anoikis via suppressing CAV-1 expression

Induction of CAV-1 expression is previously identified to be critical for anoikis resistance in various cancers [16]. In this study, we investigated the role of CAV-1 in regulating anoikis resistance in MDA-MB-231 cells and followed by the study of TBMS-V ′s action on such protein. First, it was observed that CAV-1 expression was up-regulated in cells grown in suspension compared to cells in attachment, and which was does-dependently suppressed by the treatment of TBMS-V (Fig. 3). Furthermore, it was found that the inhibitory effect of TBMS-V on anoikis resistance was restored by CAV-1 transfection, indicating that TBMS-V sensitizes anoikis via suppressing CAV-1 expression (Fig. 4).

3.3. TBMS-V sensitizes anoikis via suppressing CAV-1-dependent EGFR activation

CAV-1 is previously reported to exerts its activity against anoikis via