RESEARCH ARTICLE

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Anti-tumor Effects of IL-1β Induced TRAIL-Expressing hUCMSCs on Embelin Treated Breast Cancer Cell Lines

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Abstract

Background: Human umbilical cord mesenchymal stem cells (hUCMSCs) have high therapeutic value in cancer treatment. We have found that pre-activating hUCMSCs with IL-1β promotes tumor necrosis factor-related apoptosis inducing ligand (TRAIL) expression and facilitates anti-tumor effect. Furthermore, embelin has been found to induce apoptosis of different cancer cell lines by upregulating the expression of TRAIL receptor 1 (DR4) and TRAIL receptor 2 (DR5). This study investigated whether IL-1β induced TRAIL-expressing hUCMSCs, in combination with low-dose embelin, could further induce apoptosis in breast cancer cell lines. **Materials and Methods:** MTT assay was used to examine the cytotoxicity of embelin in MDA-MB-231 and MCF-7. To detect the interested protein expression in cells, Western blot and cell immunofluorescence were used to double-confirm the observed results. Annexin V/PI apoptosis assay was detected by flow cytometry to analyze the apoptosis rate of embelin treated breast cancer cell lines and the effect of co-culturing with breast cancer cells and hUCMSCs. **Results:** Using Western blot and immunofluorescence, we found that breast cancer cell lines treated with low-dose embelin (2.5-5 μM) increased the expression of apoptosis-related receptor DR4, DR5 and the cleaved caspase 8, 9 and 3. Moreover, TRAIL expression was enhanced in IL-1β induced hUCMSCs. Combining these observations, we expected that coculturing IL-1β induced hUCMSCs with low dose embelin treated MDA-MB-231 and MCF-7 cells might enhance the apoptosis of breast cancer cells. We confirmed via flow cytometry that coculture of IL-1β induced TRAIL-expressing hUCMSCs and embelin treated MDA-MB-231 and MCF-7 cells enhances the apoptosis rate of these breast cancer cells. **Conclusion:** We found that embelin upregulated the expression of DR4 and DR5 to increase the TRAIL-mediated apoptosis in breast cancer cell lines. Low dose embelin treated breast cancer cell lines in combination with IL-1β induced TRAIL-expressing hUCMSCs may become a potential anti-tumor therapy.

Keywords: Human umbilical cord mesenchymal stem cells (hUCMSCs)- Interleukin-1β (IL-1β)

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Introduction

Therapeutic application of Human umbilical cord mesenchymal stem cells (hUCMSCs) in cancer treatment is due to its demonstrated characteristics of low tumorigenicity, homing capability, and tumoricidal activity. Human bone marrow-derived mesenchymal stem cells (hBMMSCs) have been found to suppress tumor growth (Secchiero et al., 2010). However, hBMMSCs have also been found to transform into tumor-associated fibroblasts (TAFs) which then promote tumor development (Paunescu et al., 2011). In contrast, human umbilical cord-derived mesenchymal stem cells (hUCMSCs) do not transform to TAFs after cocultured with breast and ovarian cancer cells (Subramanian et al., 2012) (Subra. As for homing ability, evidences have indicated that injured tissues are known to be associated with releasing cytokines and growth factors such as tumor necrosis factor-alpha (TNF-α), and interleukins (IL) (Egea et al., 2011). In our previous study we have found that IL-1β could enhance hUCMSCs adhesion to endothelial cells (Wu et al., 2019), as well as increasing the transendothelial migration ability (Guo et al., 2018). These studies suggest that through the process of adhesion to endothelial cells and transendothelial migration, hUCMSCs may migrate to the inflammation sites. The migration ability of hUCMSCs to sites of tumorigenesis and their capability as efficient cellular vehicles for tumoricidal activity support the therapeutic potential for MSCs in cancer therapy. Past studies have demonstrated the tumor-suppressive effect of engineered MSCs, specifically their capability of migrating to various tumors and locally secreting

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therapeutic proteins including interferon β (IFN-β), IFN-γ and tumor necrosis factor-related apoptosis inducing ligand (TRAIL) (Li et al., 2015).

Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone), a naturally occurring benzoquinone found in the fruit of the Embelia ribes, possesses anti-inflammatory and anti-cancer properties (Xu et al., 2005). Embelin was known as a cell-permeable, small molecular weight inhibitor of X-linked inhibitor of apoptosis protein (XIAP) that releases caspase 3 and caspase 9 and initiates caspase pathways leading to apoptosis (Hussain et al., 2017). Numerous studies have shown that embelin modulates NF-kB signaling pathways and demonstrates cytotoxic effects on a variety of cancer cell lines (Ahn et al., 2007; Danquah et al., 2012). Recent investigations found that embelin enhances TRAIL-induced apoptosis through TRAIL-Receptor 1 (DR4) and TRAIL-Receptor 2 (DR5) upregulation in human leukemia cells (Hu et al., 2015).

TRAIL or Apo 2 ligand (TRAIL/Apo2L) is a type II transmembrane protein and belongs to the tumor necrosis factor (TNF) superfamily of ligands capable of initiating apoptosis by binding to its receptors. TRAIL is able to trigger apoptosis in some tumor cell lines, such as breast cancer and colon cancer (Seki et al., 2003). By using TRAIL gene-targeted mice, it has been found that TRAIL plays an important role in suppressing tumor initiation and metastasis (Cretney et al., 2002). Recombinant soluble TRAIL exhibited no detectable cytotoxicity to normal tissues both in murine and humans, and has also demonstrated its safety as a delivery vector for cancer therapeutic agents in vitro on various cell lines (Ashkenazi et al., 1999). However, the short half-life of recombinant TRAIL in serum has limited its application potential in clinical use (de Miguel et al., 2016). Therefore, a novel therapeutic approach capable of overcoming the current treatment barrier is needed.

Our previous studies show that pre-stimulating hUCMSCs with IL-1β increase protein level of TRAIL and enhance apoptosis ability on breast cancer cell lines (Liang et al., 2021). Five homologous human receptors for TRAIL have been found (Kimberley and Screaton, 2004). Both the death receptors DR4 and DR5 possess a death domain capable of transducing the apoptosis signal. Different cancers induce TRAIL-related apoptosis with different contributions of DR4 or DR5. Though colon and breast cancer cells have been found to use DR5 for apoptosis induction (Kelley et al., 2005), pancreatic cancer cell lines may trigger apoptosis primarily via DR4 (Lemke et al., 2010). TRAIL death receptors undergo constitutive apoptosis in some cancer cells, which makes them a promising target for cancer therapy. hBMMSCs have been used as delivery vectors to overcome the limitations posed by the short half-life of recombinant soluble TRAIL (Yuan et al., 2015). The prolonged half-life of TRAIL expression and the homing ability of hUCMSCs stimulated by IL-1β, make these cells excellent delivery vectors to overcome the limitations posed by the short half-life of soluble TRAIL in suppressing tumorigenicity.

It has been demonstrated that the relative sensitivity of the breast cancer cell lines to TRAIL are different. MDA-MB-231 cells were found to be a TRAIL-sensitive breast cancer cell line, but MCF-7 cells displayed a certain degree of resistance to TRAIL (Zhang and Zhang, 2008). In this study, we found that embelin enhances the expression of TRAIL receptors DR4 and DR5 on both MDA-MB-231 and MCF-7 breast cancer cell lines. Additionally, we also examined embelin regulated caspase 8, caspase 9 or caspase 3 protein levels potentially leading to different apoptosis pathways in these two cell lines. Further, we confirmed the anti-tumor ability of IL-1β induced TRAIL-expressing hUCMSCs on embelin-treated MDA-MB-231 and MCF-7 breast cancer cell lines via flow cytometry. The aim of this study was to assess whether the combination of IL-1β induced TRAIL-expressing hUCMSCs with embelin-treated breast cancer cells could enhance the apoptosis of cancer cells. Results from this study may yield potential anti-breast cancer therapeutics.

Materials and Methods

Human Umbilical Cord Mesenchymal Stem Cells (hUCMSCs)

hUCMSCs were purchased from Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan. hUCMSCs were maintained in low serum defined medium including 56% Low-glucose Dulbecco's Modified Eagle Medium (Life technology, NY, USA), 37% MCDB 201 Medium (Sigma, MO, USA), 2% fetal bovine serum (Thermo, Logan, UT), 0.5 mg/ml AlbuMAX® I (Life technology, NY, USA), 1X Insulin-Transferrin-Selenium-A (Life technology, NY, USA), 10 nM Dexamethasone (Sigma, MO, USA), 10ng/ml Epidermal growth factor (PeproTech, NJ, USA), 50nM L-ascorbic acid 2-phosphate (Sigma, MO, USA), and 1 ng/ml of platelet-derived growth factor-BB (PeproTech, NJ, USA). Cells were incubated at 37°C and 5% CO_2. When cells reached 70-80% confluence, the cells were detached with HyQtase (Thermo, Logan, UT) and replated in culture dishes at a ratio of 1:4.

Breast Cancer Cells

Human breast cancer cells MDA-MB-231, MCF-7 (purchased from BCRC, Hsinchu, Taiwan) were maintained in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Life technology, NY, USA) consisting with 10% fetal bovine serum, and 1% HyClone® Penicillin-Streptomycin solution (Thermo, Logan, UT). Cells were incubated at 37°C and 5% CO_2. When cells reached 70-80% confluence, the cells were detached with HyQtase (Thermo, Logan, UT) and replated in culture dishes at a ratio of 1:4.

MTT assay

To examine the cytotoxicity of embelin on hUCMSCs, 1×10^4 cells per well were seeded in 96-well plates with 2% culture medium (2% CM; DMEM (Low glucose) containing 2% FBS). Cells were treated with embelin at various concentrations (0-200μM) for 24 hrs. Following incubation, 1 mg/ml MTT reagent was added to each well for 4 hrs at 37°C. The cells were then suspended in dimethyl sulfoxide for 2 hrs at 37°C and detected using

multimode microplate readers (Infinite 200, TECAN) at a wavelength of 545 nm.

Annexin V/PI apoptosis assay

Cells were collected and pelleted at 1000 rpm for 5 mins and washed twice with 1 mL PBS. The resulting pellets were resuspended in 100 µl of 1X Annexin V binding buffer and stained with 50 µg/mL annexin V-FITC and 100 µg/mL propidium iodide (Life, NY, USA). Samples were kept at room temperature (RT) for 20 mins and protected from light. After the incubation period, samples were analyzed immediately using flow cytometry (Beckman, IL, USA).

Western Blotting

Cells were washed with 1X PBS and lysed in M-PER Mammalian Protein Extraction Reagent (Thermo, IL, USA) with 1% Halt Protease Inhibitor Cocktail (Thermo, IL, USA). The extractions were gently shaken for 3 mins and centrifuged at 14,000 g for 10 mins at 4oC. Protein concentrations were determined by Coomassie Plus (Bradford) Protein Assay reagent (Thermo, IL, USA) and Multimode microplate readers (Infinite 200, TECAN). Protein samples were electrophoresed on 10% or 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then transferred to polyvinylidene fluoride membranes. Membranes were blocked for 1 hour at RT using 5% Fish Gelatin Blocking Buffer (AMRESCO, OH, USA) in TBST buffer (136mM NaCl, 2.7mM KCl, 25mM Tris-base, 0.05% Tween-20). After blocking, membranes were incubated with primary antibody TRAIL R1, TRAIL R2 (Novus, St Charles, USA), caspase 8 (GeneTex, CA, USA), caspase 9 and caspase 3 (Cell Signaling, MA, USA) diluted at 1:1,000 and beta-actin antibody (GeneTex, CA, USA) diluted at 1:10,000 in TBST buffer at 4°C overnight. Then membranes were washed three times with TBST, and incubated with Rabbit IgG antibody (GeneTex, CA, USA) for 1 hour at RT. The results were detected by LAS-4000 Luminescence Imaging System (GE, CT, USA) and analyzed by AlphaEaseFC 4.0 software.

Cell Immunofluorescence and Images

Cancer cells were plated in 24-well plates and starved for 12-16 hours in DMEM F12 containing 0.5% FBS. After starvation, cells were treated with different concentrations of embelin for 24 hours. Cells were fixed with PBS containing 4% (v/v) paraformaldehyde for 15 mins and permeabilized with 0.1% Triton X-100 in PBS for 10 mins. Cells were then blocked with 2% BSA in PBS for 30 mins and incubated with primary antibody at 4oC overnight. After cells were washed three times with PBS, they were incubated with appropriate second antibodies for 1 hour at RT. Cells were stained with Hoechst 33258 (Sigma, MO, USA), washed three times with PBS and imaged using a fluorescent microscope (DM6000B, Leica, German).

Pre-stimulation of hUCMSCs with Cytokines

Before coculture, hUCMSCs were detached and plated at $1x10^5$ cells in 2% culture medium (2%CM; DMEM (low glucose) containing 2% fetal bovine serum) in 6-well plates and incubated for 4-6 hrs. For pre-activation, hUCMSCs were incubated in 2% CM containing 100 ng/ml IL-1β (PeproTech, NJ, USA).

Direct Co-culture of hUCMSCs and Breast Cancer Cells

MDA-MB-231 or MCF-7 cells were pretreated with different concentrations of embelin for 24 hrs. After pretreatment, an equal number of breast cancer cells were added to hUCMSCs containing wells for direct coculture for 24 hours with embelin in 2% CM. Both types of cells were detached and collected into the same tubes. The mixtures were washed with PBS by centrifugation, suspended in 100µl PBS and labeled with CD90-PE (Beckman Coulter, France) for 45 mins on ice in order to distinguish hUCMSCs and breast cancer cells by flow cytometry.

Statistical Analysis

Quantitation data was analyzed by the student's t-test and one-way ANOVA. P values <0.05 were considered statistically significant. Statistical analysis was performed with Prism 5 software.

Results

Effect of embelin on MDA-MB-231 and MCF-7 cell apoptosis

To investigate the effect of embelin on apoptosis of MDA-MB-231 and MCF-7 breast cancer cell lines, cells were treated with 2.5, 5, 10, 50 µM of embelin for 24 hrs and the extent of apoptosis was quantified using flow cytometry. As shown in figure 1, there was no significant increase in apoptotic rate in both cell lines following treatment with 2.5, 5, 10 and 50 µM embelin for 24 hrs. In these experiments, the DR4 and DR5 ligand recombinant human TRAIL (rhTRAIL) induce the apoptosis of both cell lines. However, 2.5, 5, and 10 µM embelin treated for 24 hours did not induce the apoptosis rate of both cell lines (Figure 1). By contrast, treatment with 50 µM embelin resulted in an apoptosis rate of ~50% in MDA-MB-231 cells and ~25% in MCF-7 cells (Figure 1b, 1d).

Treatment of MDA-MB-231 and MCF-7 with embelin enhances DR4 and DR5 expression

In light of the fact that DR4 and DR5 expression play important roles in many TRAIL-related cancer cells apoptosis, we analyzed the protein levels of DR4 and DR5 through western blotting after 24 hours treatment with embelin. The results showed that both DR4 and DR5 protein expression were upregulated in MDA-MB-231 cells at 5µM and 10µM embelin treatment; there was no significant change at the concentration of 2.5µM (Figure 2a, b, d, e). We further examined surface expression of DR4 and DR5 on cell membrane by immunofluorescence. The results are consistent with the Western blot data that embelin treatment enhances DR4 and DR5 expression in MDA-MB-231 cells (Figure 2c, f). The expression of DR4 in MCF-7 cells increased significantly at 2.5 and 10µM of embelin treatment the same as in MDA-MB-231 cells (Figure 2g, h). Interestingly, in MCF-7 cells DR5 showed significant increase only at embelin concentrations of

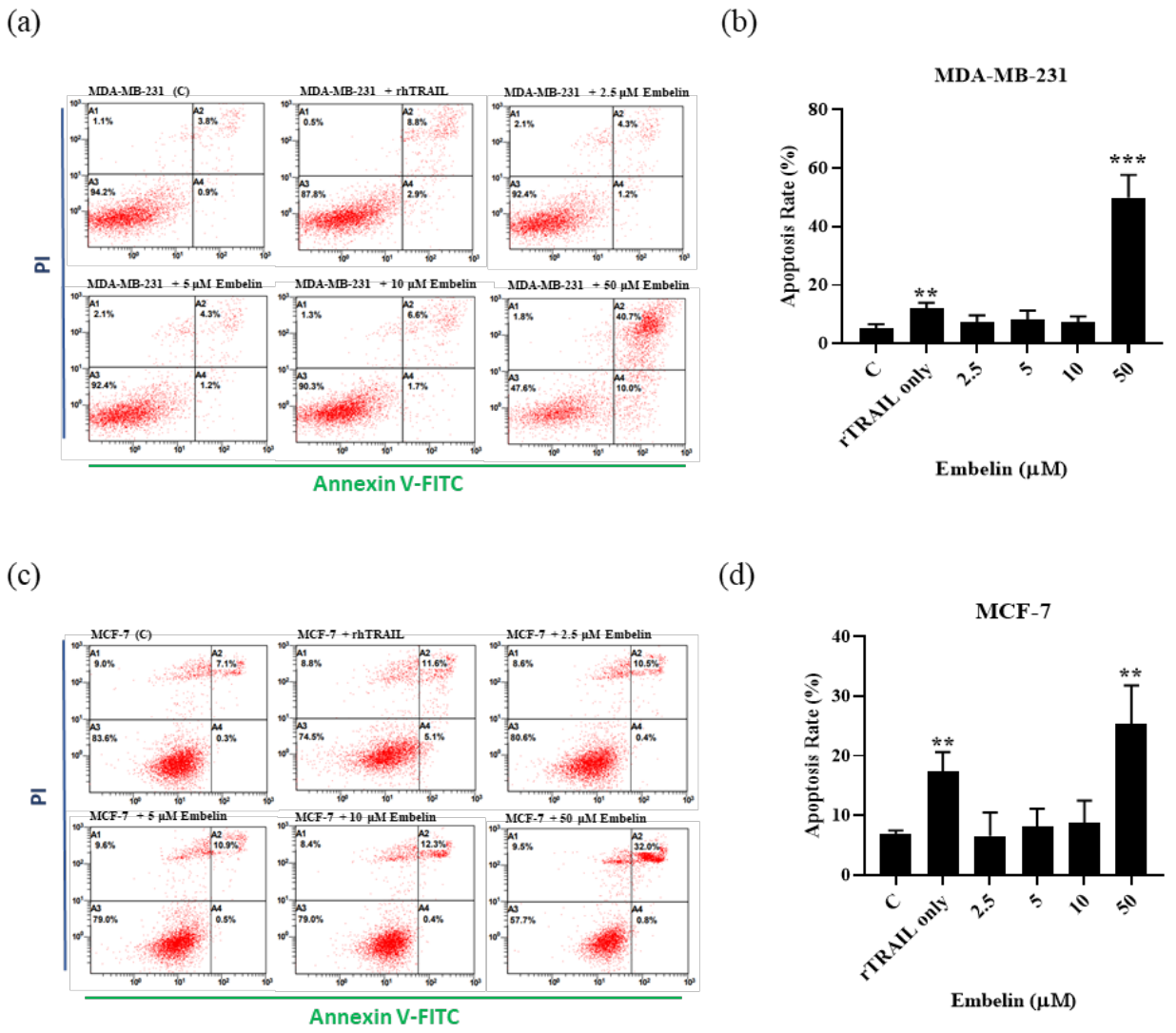

Figure 1. Apoptosis Induced by Embelin in MDA-MB-231 and MCF-7 Cells. Annexin V/propidium iodide (PI) dye was used to determine the rate of apoptosis following treatment of (a) MDA-MB-231 and (b) MCF-7 cells with different concentrations of Embelin for 24hrs and statistically analysis (b and d). Breast cancer cells treated with 100 ng/ml rhTRAIL are regarded as the positive control. The data were represented as mean ± SD (n=3, *P<0.05, **P<0.01, *** P<0.001).

2.5μM (Figure 2j, k). The immunofluorescence results are consistent with the Western blot data that low-dose embelin treatment enhances DR4 and DR5 expression in MCF-7 cell lines (Figure 2i, l).

Embelin induces activation of caspase proteins in breast cancer cells

To determine whether caspase activation was involved in embelin-treated breast cancer cells, we next examined the activation of caspase 8, caspase 9, the initiator of the intrinsic and extrinsic apoptosis pathways and caspase 3. MDA-MB-231 and MCF-7 were incubated with different concentrations of embelin (0, 2.5, 5, 10 μM) for 24 hrs. The activation of caspase 8, 9 and 3 were analyzed by western blotting. The results showed that cleaved caspase 8, cleaved caspase 9 and cleaved caspase 3 increased significantly in both MDA-MB-231 and MCF-7 treating with embelin at 5 μM and 10 μM (Figure 3). These results suggest that low-dose (5 μM and 10 μM) embelin-treated breast cancer cell lines lead to apoptosis through both intrinsic or extrinsic pathways.

IL-1β-stimulated hUCMSCs induce apoptosis in embelin-treated MDA-MB-231 and MCF-7

In our previous study, we found that IL-1β (100 ng/ml) stimulation can induce TRAIL expression in hUCMSCs. To examine the effect of IL-1β induced TRAIL-expressing hUCMSCs on breast cancer cell apoptosis and whether treatment with embelin could further enhance apoptosis in breast cancer cell lines. MDA-MB-231 and MCF-7 cells were pre-treated with embelin of 5 and 10 μM for 24 hrs, then cells were directly co-cultured with naïve hUCMSCs or IL-1β induced TRAIL-expressing hUCMSCs with/without embelin for 24 hrs. Treatment with 5 or 10 μM embelin on hUCMSCs for 24 hours, the results showed no cytotoxic effect on hUCMSCs (Sl Figure). Cocultured 5 or 10 μM embelin pre-treated MDA-MB-231 cells with IL-1β induced TRAIL-expressing hUCMSCs,

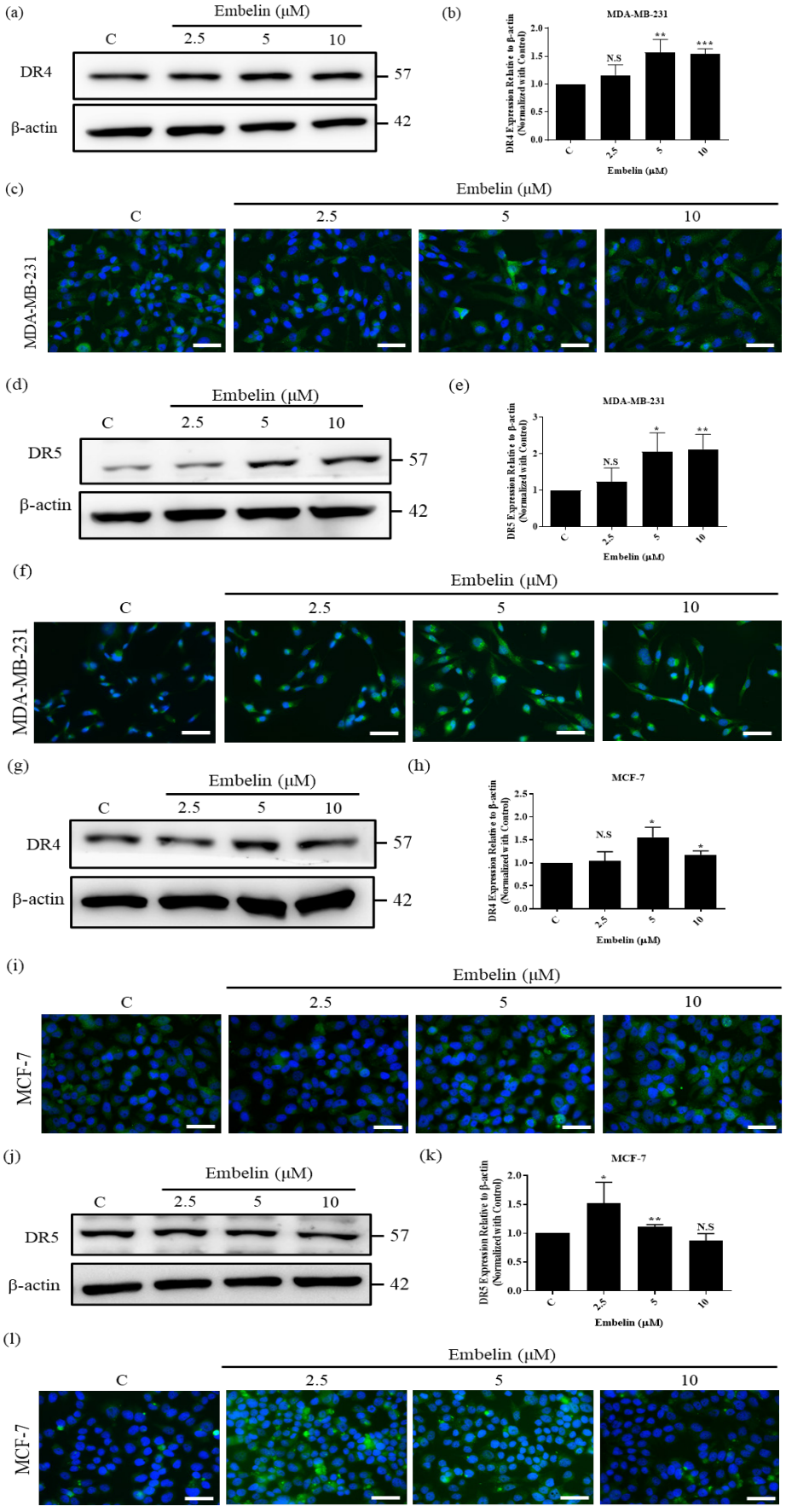

Figure 2. Embelin Upregulated DR4 and DR5 Expressions. (a, d) MDA-MB-231 and (g, j) MCF-7 cells were incubated with embelin of 2.5, 5 and 10 μM for 24 hrs, and DR4 and DR5 protein expressions were determined by Western blot. Quantify of western blot of DR4, DR5 in (b, e) MDA-MB-231 and (h, k) in MCF-7. The data are presented as the mean ± SD of three independent experiments. (n=3, *P<0.05, **P<0.01, *** P<0.001). Immunofluorescence staining for DR4, DR5 (green) and DAPI (blue) in (c, f) MDA-MB-231 and (d, l) MCF-7 cells after treatment with embelin of 2.5 to 10 μM for 24 hrs (Scale bar = 50μM).

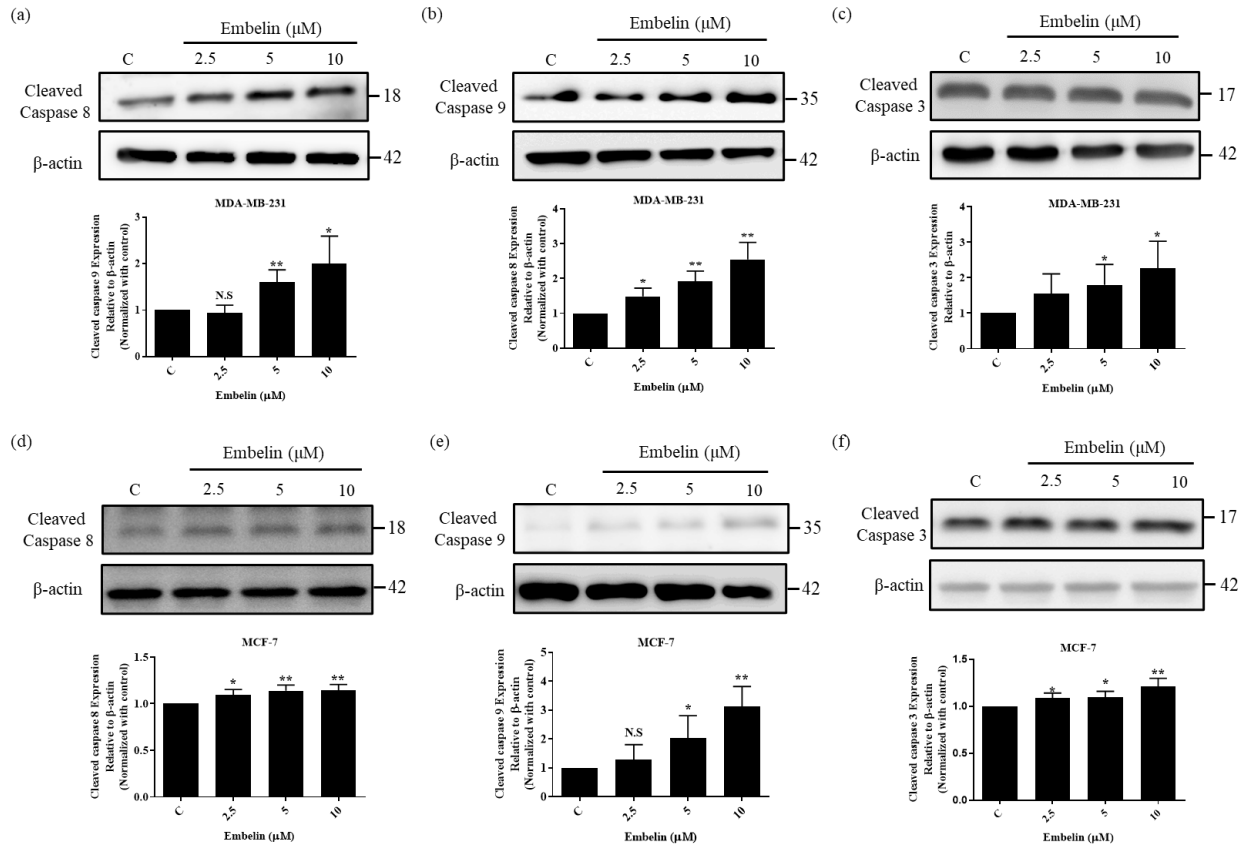

Figure 3. Embelin Induces Activation of Caspase Proteins in Breast Cancer Cells. Following treatment of breast cancer cells with different doses of embelin (0, 2.5, 5 and 10 μM) for 24hrs, Western blotting was used to analyze the expression of cleaved caspase 8, cleaved caspase 9, cleaved caspase 3 and quantitative graphs of (a, b, c) MDA-MB-231 cells and (d, e, f) MCF-7 cells. (n=3, *P<0.05, **P<0.01, *** P<0.001).

the apoptosis rate of the MDA-MB-231 cells was increased (Figure 4a, b). However, co-cultured the naïve hUCMSCs with 5 μM embelin treated MDA-MB-231, compared with control, the apoptosis rate did not change (Figure 4b). The apoptosis rate of 5 or 10 μM embelin treated MDA-MB-231 cells co-cultured with IL-1β induced TRAIL-expressing hUCMSCs is significantly higher than co-cultured with the naïve hUCMSCs (Figure 4a-d). As for 5 μM embelin pre-treated MCF-7 cells, co-culturing with naïve hUCMSCs or IL-1β induced TRAIL-expressing hUCMSCs and 5μM embelin did not alter the apoptosis rate in comparison to MCF-7 cells only. However, 10 μM embelin pre-treated MCF-7 cells co-cultured with IL-1β induced TRAIL-expressing hUCMSCs, the apoptosis rate was increased significantly (Figure 4g, h). These results showed that the apoptosis of 10 μM embelin pre-treated MDA-MB-231 and MCF-7 cells can be enhanced by co-culture with IL-1β induced TRAIL-expressing hUCMSCs for 24 hrs.

Discussion

TRAIL is a member of the TNF family proteins, capable of inducing apoptosis in most cancer cells but not in normal cells (Daniels et al., 2005). TRAIL induces apoptosis by activating cleaved caspase 8, cleaved caspase-9 and BAX in TRAIL-sensitive cancer cells (Johnstone et al., 2008). Previous studies indicated that TRAIL-resistant cancer cell lines expressed NF-kB in nuclear extracts, which could inhibit apoptosis induced by its death receptor's action (Keane et al., 2000).

Embelin (25-50 μM) has been found to inhibit cell proliferation and induce apoptosis in several human cancer cells (Siegelin et al., 2009; Park et al., 2015; Hussain et al., 2017). Low dose (1.5-5μM) embelin can enhance the sensitivity of renal cancer cell lines to an anti-renal cancer agent-axitinib by inhibiting the HIF pathway (Fang et al., 2023). Recently, we found that the antitumor effect of embelin (in a concentration of 50 μM) in breast cancer cell lines occurred via downregulating cellular FADD-like IL-1ß-converting enzyme inhibitory protein (cFLIP), a regulator in TRAIL-mediated apoptosis, which is correlated with TRAIL resistance in cancer cells (Liang et al., 2021). Low-toxicity embelin has also been found to enhance TRAIL-induced apoptosis via DR4 and DR5 upregulation and caspase activation in human leukemia HL-60 cells (Hu et al., 2015). Many drugs have been found to sensitize tumor cells to TRAIL by DR4 and/or DR5 upregulation. Doxorubicin and etoposide have been found to increase DR4 expression on small cell lung carcinoma cells to sensitize TRAIL induced apoptotic effect of TRAIL through increasing DR5 on cell surfaces (Vaculova et al., 2010). Treatment with cisplatin led to the upregulation of DR5 in glioblastoma-derived stem cells and restored TRAIL apoptotic pathway in the neurospheres (Ding et al., 2011). While research on embelin is ongoing,

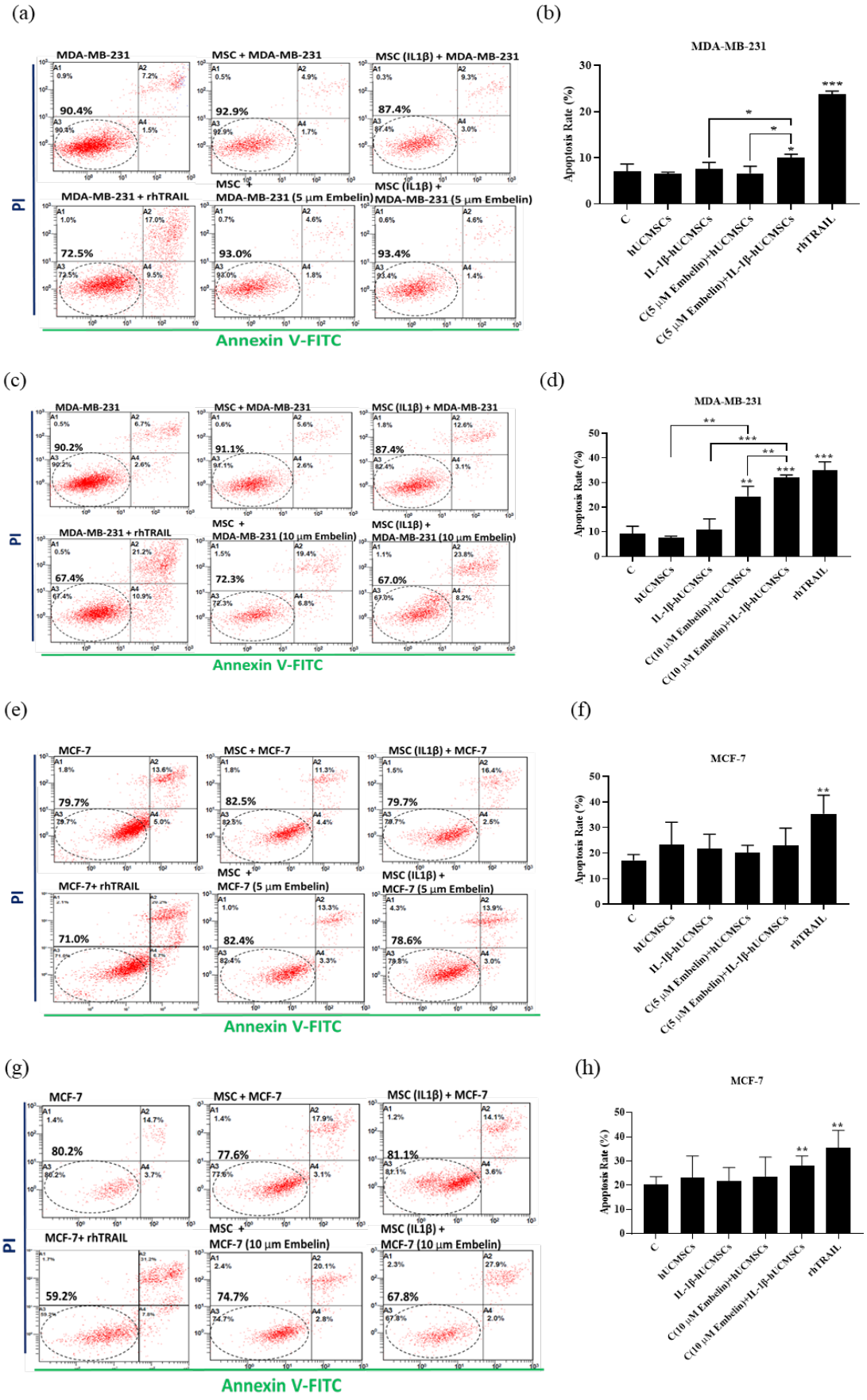

Figure 4. Stimulated hUCMSCs Combined with Embelin Induce Apoptosis in Breast Cancer Cell Lines. After co-culture of naïve hUCMSCs or IL-1β induced TRAIL-expressing hUCMSCs (IL-1β-hUCMSCs) with 5 and 10 μM embelin treated MDA-MB-231 or MCF-7 cells for 24hrs, cells were collected and labeled with anti-CD90 antibody to distinguish hUCMSCs from breast cancer cells. Flow cytometry was used to analyze the apoptosis rate in (a, b, c, d) MDA-MB-231 and (e, f, g, h) MCF-7 by staining with Annexin V-FITC and PI. (n=3, *P<0.05, **P<0.01, *** P<0.001).

we focused on two common breast cancer cell lines including MDA-MB-231 (TRAIL-sensitive) and MCF-7 (TRAIL-resistant) in this study to elucidate the weather embelin-induced apoptosis in human breast cancer cells involves DR4 and DR5 upregulation. In our analysis, we demonstrated that low dose embelin (5 μM) upregulated both DR4 and DR5 expression in TRAIL sensitive and TRAIL resistant breast cancer cell lines using western blotting and Immunofluorescence staining.

There are two main signaling pathways that trigger apoptosis, the intrinsic pathway and extrinsic death receptor pathway (Igney and Krammer, 2002). In the present study, we found that after MDA-MB-231 and MCF-7 cells were treated with different doses of embelin for 24hrs, both cleaved caspase 8, cleaved caspase 9 and cleaved caspase 3 levels increased significantly. These results indicate embelin-induced apoptosis in these two cancer cell lines through both the extrinsic and intrinsic apoptosis pathways.

Given its ability to induce the apoptosis of cancer cells selectively, rhTRAIL has been used as anti-cancer therapy. However, the efficacy was poor (Herbst et al., 2010; Quintavalle and Condorelli, 2012). Our previous studies found that stimulated hUCMSCs with IL-1β increased TRAIL expression (Liang et al., 2021). Pre-stimulated hUCMSCs with IL-1β induced TRAIL can overcome the short half-life when compared with rhTRAIL. In addition to the limitation of short half-life, the resistance to TRAIL-induced apoptosis may also be involved in poor efficacy of TRAIL anti-cancer treatment. Low dose embelin induced DR4 and DR5 may be a good strategy to improve the TRAIL induced apoptosis of cancer cells. In this study, we found that the apoptotic rate of MDA-MB-231 cells increased during co-culture with IL-1β induced TRAIL-expressing hUCMSCs. Most importantly, a combination of low-toxicity embelin with IL-1β induced TRAIL-expressing hUCMSCs could enhance the apoptosis rate of MDA-MB-231 and MCF-7 cells. Moreover, cytokines secreted by cancer cells enhance the homing ability of hUCMSCs, which could improve the clinical efficacy of stem cell therapy (Kang et al., 2012). We assumed that IL-1β induced homing ability and TRAIL expression, suggesting that IL-1β may be a key molecule to modulate hUCMSCs in inhibiting the growth of cancer cells.

In summary, our data indicated that low dose embelin promoted DR4 and DR5 expression in breast cancer cell lines. IL-1β induced TRAIL-expressing hUCMSCs combined with embelin can enhance apoptosis more significantly in TRAIL-sensitive (MDA-MB-231) than in TRAIL-resistant (MCF-7) cell lines. As a result, we suggest that low dose embelin combined with IL-1βinduced TRAIL-expressing hUCMSCs may possess the advantage of homing ability and increased clinical efficacy in TRAIL-related cancer therapies. These results suggest a different path for the clinical use of mesenchymal stem cells in cancer therapy.

Author Contribution Statement

H-SW contributed to conception and design, development of methodology. J-WT, EH, Y-HL, Y-HC, Y-SC contributed to acquisition of data. J-MW, Y-ST and H-SW contributed to material support and interpretation of data. J-WT, Y-HL and H-SW contributed to writing of the manuscript. Part of the data are from J-WT's master thesis. All authors read and approved the final manuscript.

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Ethics Approval and Consent to Participate

Not applicable.

Data Availability

All data needed to evaluate the conclusions in the paper are present in the paper.

Conflicts of Interest

The authors declare no conflicts of interest.

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