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Zebularine and trichostatin A sensitized human breast adenocarcinoma cells towards tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis

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ABSTRACT

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising cancer therapeutic agent due to its selective killing on cancer cells while sparing the normal cells. Nevertheless, breast adenocarcinoma cells can develop TRAIL resistance. Therefore, this project investigated the anti-cancer effects of the combination of epigenetic drugs zebularine and trichostatin A (ZT) with TRAIL (TZT) on the human breast adenocarcinoma cells. This treatment regimen was compared with the natural anti-cancer compound curcumin (Cur) and standard chemotherapeutic drug doxorubicin (Dox). As compared to TRAIL treatment, TZT treatment hampered the cell viability of human breast adenocarcinoma cells MDA-MB-231 significantly but not MCF-7 and immortalized non-cancerous human breast epithelial cells MCF10A. Unlike TZT, Cur and Dox treatments reduced cell viability in both human breast adenocarcinoma and epithelial cells significantly. Nevertheless, there were no changes in cell cycle in both TRAIL and TZT treatments in breast adenocarcinoma and normal epithelial cells. Intriguingly, Cur and Dox treatment generally induced G2/M arrest in MDA-MB-231, MCF-7 and MCF10A but Cur induced S phase arrest in MCF10A. The features of apoptosis such as morphological changes, apoptotic activity and the expression of cleaved poly (ADP) ribose polymerase (PARP) protein were more prominent in TRAIL and TZT-treated MDA-MB-231 as compared to MCF10A at 24 h post-treatment. Compared to TZT treatment, Cur and Dox treatments exhibited lesser apoptotic features in MDA-MB-231. Collectively, the sensitization using Zeb and TSA to augment TRAIL-induced apoptosis might be an alternative therapy towards human breast adenocarcinoma cells, without harming the normal human breast epithelial cells.

1. Introduction

Breast cancer is the most frequently diagnosed and the leading cause of cancer mortality among females (Bray et al., 2018). With this, highly aggressive triple-negative breast cancer (TNBC) which lacks estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) constituted about 20% of all breast cancer cases worldwide (Badve et al., 2010; Wahba and El-Hadaad, 2015). Currently, chemotherapy is the main therapeutic option to improve the clinical outcome of TNBC patients. Nevertheless, prognosis remains poor due to the high recurrence rate and short disease-free survival rate (Wahba and El-Hadaad, 2015). Hence, it is imperative to search for alternative therapy to combat breast cancer.

Belongs to tumour necrosis factor (TNF) family members, TNF-related

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apoptosis-inducing ligand (TRAIL) is a 20 kDa type II transmembrane protein which exerts its tumour surveillance by regulating extrinsic and intrinsic apoptotic pathways (Falschlehner et al., 2007). Upon engagement to death receptors such as death receptor 4 (DR4) and death receptor 5 (DR5), Fas-associated protein with death domain (FADD) and caspase 8/10 are recruited, forming death-inducing signalling complex (DISC) (Ashkenazi, 2002; Riedl and Shi, 2004; Lemke et al., 2014). This leads to caspase 8 auto-activation and subsequently results in caspase cascade signalling that eventually causes apoptosis (Johnstone et al., 2008). In the intrinsic pathway, activated caspase 8 cleaves Bcl-2 homology domain 3 interacting-domain death agonist (Bid) which causes the activation of pro-apoptotic proteins Bax and Bak and causing the permeabilization of the mitochondrial membrane. This directs the activation of caspase 3/9 and causes apoptosis (Wang, 2008; De Miguel et al., 2016).

Although demonstrating tumour surveillance properties, many cancers develop defensive mechanisms towards TRAIL such as the downregulation of death receptors (Rahman et al., 2009; Zhang et al., 2009), overexpression of anti-apoptotic proteins (Cao et al., 2004; Allensworth et al., 2012; Riley et al., 2013) and the dysregulation of caspase activities (Wu et al., 2010). With respect to this, clinical studies targeting TRAIL apoptotic pathways exhibited no improved clinical outcome among patients (Bellail et al., 2009; Lemke et al., 2014). These had caused the neglection of TRAIL as an anti-cancer agent. However, TRAIL is worth for investigation due to its selective killing of cancer cells but not normal cells (Ashkenazi, 2002; Holland, 2013). Therefore, combinational therapy is developed by using sensitizers to reverse TRAIL resistance and maximize the anti-cancer potentials towards cancerous cells.

Zebularine (Zeb) is a DNA methyltransferase (DNMT) inhibitor which belongs to nucleoside analogues (Yoo et al., 2004; Mani and Herceg, 2010; Wu et al., 2019). It inhibits the DNMT activities through its engagement to DNMT and hinders their methyl transfer activities (Zhou et al., 2002). The association of Zeb and TRAIL is well illustrated from the previous research. Zeb increased the fucosylation of death receptor and augmented TRAIL-induced apoptosis in cancerous cells (Moriwaki et al., 2010). Furthermore, trichostatin A (TSA) is a histone deacetylase (HDAC) inhibitor which consists of chemical group hydroxamic acid that impedes HDAC activities (Bolden et al., 2006; Halsall and Turner, 2016). Besides inhibiting HDAC activities, TSA caused cell cycle arrest and apoptosis in many cancerous cells (Bolden et al., 2006). TSA was proved to augment the TRAIL-induced apoptosis in cancerous cells through the downregulation of anti-apoptotic proteins such as cellular FLICE-like proteins (cFLIPs) (Mühlethaler-Mottet et al., 2006; Park et al., 2009). Recently, research performed by Kaminskyy et al. (2011) demonstrated that co-administration of DNMT and HDAC inhibitors sensitized the non-small cell lung cancer towards TRAIL-induced apoptosis via the restoration of caspase 8 activities. This evidence provided the notion that administration of DNMT and HDAC inhibitors collectively sensitized the cancerous cells towards TRAIL-induced apoptosis.

In this study, we aimed to investigate the anti-cancer effects of epigenetic drugs Zeb and TSA as sensitizers to human breast adenocarcinoma cells towards TRAIL-induced apoptosis. Besides, this treatment will be compared with the natural anti-cancer compound curcumin (Cur) and standard chemotherapeutic drug doxorubicin (Dox).

2. Materials and methods

2.1. Chemicals

Zebularine (Zeb) (Sigma, USA) and trichostatin A (TSA) (Sigma, USA) were dissolved and further diluted in dimethyl sulfoxide (DMSO) (Sigma, USA). Recombinant human TRAIL/TNFSF10 protein (TRAIL) (375-TL-010, R&D System) was dissolved in the 1X phosphate buffered saline (PBS) and 0.1% bovine serum albumin (BSA). Curcumin (Cur) (Sigma, USA) was dissolved in the absolute ethanol and further diluted with Dulbecco's Modified Eagle Medium (DMEM) (Cellgro, USA). Doxorubicin (Dox) (D1515, Sigma) was dissolved in sterile water.

2.2. Cell culture and maintenance

Human breast adenocarcinoma cells MDA-MB-231, MCF7 and human breast epithelial cells MCF10A were purchased from America Type Culture Collection (ATCC). Both human breast adenocarcinoma cells were cultured in DMEM and supplemented with 10% fetal bovine serum (FBS) (Sigma, USA) and 1% penicillin-streptomycin (Pen-Strep) (Gibco, USA). MCF10A was maintained in MCF10A complete media which consisted of DMEM/F12 (Cellgro, USA) and supplemented with 5% horse serum (Gibco, USA), 20 ng/μL of human epidermal growth factor (hEGF) (Sigma, USA), 0.5 μg/mL of hydrocortisone (Nacalai Tesque, Japan), 10 mg/mL of insulin (Sigma, USA) and 1% Pen-Strep as described in previous studies (Chung et al., 2017). MCF10A was starved with MCF10A complete media without 20 ng/μL hEGF one day prior to any experiment. The cells were maintained in an incubator at 37°C and supplied with 5% carbon dioxide.

2.3. Cell viability assays

MDA-MB-231, MCF-7 and MCF10A (5000 cells/well) were seeded and allowed for attachment overnight. After the treatments, the cells were subjected to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays to determine the percentage of cell viability based on previous studies (Al-Khdhairawi et al., 2017; Chung et al., 2017; Mai et al., 2018).

2.4. Morphological assessment through histochemical staining

The untreated and treated cells were stained in hematoxylin and eosin dyes (Fisher Scientific, USA) as described previously (Abubakar et al., 2016; Lim et al., 2013). After preparing the slides, the cellular morphological images were captured under x40 magnification using Nikon 80i Eclipse microscope (Nikon, Japan).

2.5. Flow cytometry analysis

The change in the cell cycle phase was evaluated by flow cytometric propidium iodide (PI) staining. After fixing the cells using pre-chilled 70% ethanol for a week, the cells were stained with PI staining solution which consisted of 0.1% Triton-X (Nacalai Tesque, Japan), 100 μg/mL RNase A (Nacalai Tesque, Japan) and 50 μg/mL PI (Nacalai Tesque, Japan). After incubation in the dark for 30 min, the cell cycle analysis was performed using BD Accuri C6 flow cytometer (BD Biosciences, USA). At least 10,000 events were examined for each sample and the data were analysed using ModFit 5.0 program.

For apoptotic activity analysis, the cells were stained with Annexin V and PI using fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kit I (BD Biosciences, USA) following the manufacturer's protocol. The apoptotic activity was quantified as the percentage of Annexin V positive and PI negative cells using BD Accuri C6 flow cytometer (BD Biosciences, USA) and at least 10,000 events were examined for each sample.

2.6. Western blot analysis of poly (ADP-ribose) polymerase (PARP) expression

The cellular lysate was prepared using ice-cold lysis buffer consisting of 1% NP40, 1 mM DTT and protease inhibitors in PBS. The protein concentration was quantified through Bradford assays (Bio-Rad Laboratories, USA). Protein (50 μg) from each sample was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting as described previously (Soo et al., 2017). Primary antibodies targeting PARP at 1: 1000 (Cell Signalling Technology, USA) and β-actin at 1: 1000 (Santa Cruz Biotechnology, USA) were

incubated on polyvinylidene difluoride (PVDF) membrane overnight. Following 3 times washing with PBST consisting of 1× PBS and 0.1% Tween-20, the blots were incubated with diluted enzyme-linked secondary antibodies. The detection of specific antibodies was carried out with Enhance Chemiluminescence (ECL)™ Select Western Blotting Detection Reagent (Sigma-Aldrich, USA). The images were captured using the ChemiDoc™ XRS + System (Bio-Rad Laboratories, USA).

2.7. Statistical analysis

All results were presented as mean ± standard error mean (SEM) in triplicates from at least three independent experiments unless stated otherwise. Statistical significance was determined using one-way ANOVA with Tukey post-hoc test through IBM SPSS Statistics 24. The statistical difference was determined by *$p < 0.05$ and **$p < 0.01$ vs control as well as #$p < 0.05$ and ##$p < 0.01$ vs TRAIL-treated cells.

3. Results

3.1. Anti-cancer potential of Zeb, TSA and TRAIL towards human breast adenocarcinoma cells and breast epithelial cells

We investigated the potential of Zeb and TSA in sensitizing the human breast adenocarcinoma cells towards TRAIL treatment. To determine the suitable inhibitory concentrations (ICs), dose responses were determined through a series of MTT assays. The treatment regimens used were IC_{25} of Zeb (4.75 μM), IC_{25} of TSA (25 nM), IC_{25} of Zeb and TSA (Termed ZT) (4 μM Zeb and 12.5 nM TSA), 50 ng/mL TRAIL and IC_{25} of ZT followed by 50 ng/mL TRAIL (Termed TZT) (See in Supplementary Fig. 1). The positive controls were IC_{50} of Cur (20 μM) and IC_{50} of Dox (0.8 μM) (See in Supplementary Fig. 2).

At 24 h post-treatment, Zeb, TSA and ZT caused no significant reduction in the cell viability towards MDA-MB-231, MCF7 and MCF10A (See in Supplementary Fig. 3). Based on Fig. 1A, ZT and TRAIL treatments did not reduce cell viability in MDA-MB-231 significantly. Nevertheless, TZT treatment exerted significant ($p < 0.05$) reduction in the cell viability of MDA-MB-231 (63.79 ± 7.93%) as compared to TRAIL treatment (111.17 ± 6.14%), suggesting administration of ZT sensitized MDA-MB-231 towards TRAIL. Same as MDA-MB-231, TZT treatment diminished the cell viability (42.95 ± 4.72%) significantly ($p < 0.05$) as compared to untreated control in MCF7. However, statistical analysis revealed no significant difference in cell viability of MCF7 between TRAIL and TZT treatments, suggesting ZT had no sensitization effects towards TRAIL treatment in MCF7. Furthermore, ZT, TRAIL and TZT treatments did not decrease the cell viability in MCF10A, suggesting ZT, TRAIL and TZT are safe towards normal cells. As compared to TZT treatment, Cur and Dox treatments decreased the cell viability of MDA-MB-231, MCF7 and MCF10A after 24 h treatment (Fig. 1A).

Following cell viability studies, we further investigated whether these effects were caused by cell cycle arrest in MDA-MB-231, MCF7 and MCF10A. Based on Fig. 1B and C and Supplementary Fig. 4A and B, there were no significant changes in the cell cycle phases of MDA-MB-231 under the treatment of Zeb, TSA, ZT, TRAIL and TZT at 24 h post-treatment. Same effects were observed in MCF7 and MCF10A under the treatments of Zeb, TSA, ZT, TRAIL and TZT (See Supplementary Fig. 4C to F). These indicated that the diminished cell viability was driven by other mechanisms. Conversely, Cur treatment resulted in G2/M cell cycle arrest in MDA-MB-231, MCF7 and S cell cycle arrest in MCF10A. Besides, Dox treatment induced very significant ($p < 0.01$) G2/M cell cycle arrest in MDA-MB-231, MCF7 and MCF10A at 24 h post-treatment (See in Supplementary Fig. 4). This suggested that the reduction of cell viability of MDA-MB-231, MCF7 and MCF10A under treatment of Cur and Dox was regulated by cell cycle arrest mechanism.

3.2. Apoptotic activities of human breast adenocarcinoma cells and breast epithelial cells after the treatment of Zeb, TSA and TRAIL

After the cell viability and cell cycle analysis, the apoptotic activities of these treatments were evaluated through numerous apoptotic analysis. Since the cell viability and cell cycle results of ZT treatment were insignificant, the apoptotic analysis was conducted based on TRAIL, TZT, Cur and Dox treatments targeting MDA-MB-231 and MCF10A. Based on Fig. 2Ai and B, TZT treatment induced very significant ($p < 0.01$) apoptotic activities (48.03 ± 6.24%) in MDA-MB-231 as compared to TRAIL treatment alone (22.82 ± 6.24%) at 24 h post-treatment. This implies that the administration of ZT augmented TRAIL-induced apoptosis in MDA-MB-231. In concordance with that, morphological assessment through histochemical staining exhibited apoptotic features such as nuclear chromatin condensation and cell shrinkage in both TRAIL- and TZT-treated MDA-MB-231 as shown in Fig. 2C. As an indicator of apoptotic activities, cleavage of PARP expression was studied through Western blot analysis. Based on Fig. 2D and Supplementary Fig. 5A, TRAIL- and TZT-treated MDA-MB-231 revealed a cleavage of PARP expression. Furthermore, apoptotic activities were rather subtle in MDA-MB-231 treated with Cur (10.35 ± 3.21%) and Dox (15.91 ± 1.77%) as compared to TZT treatment (48.03 ± 6.24%). Although resulted apoptotic features such as chromatin condensation and cell shrinkage, Cur and Dox treatments caused minimal expression of PARP cleavage as compared to TZT treatment.

The apoptotic activities induced by TRAIL and TZT treatment were further evaluated in MCF10A as shown in Fig. 2Aii and B. In concordance with the cell viability studies, TRAIL and TZT treatments resulted in very minute apoptotic activities in MCF10A. These results were supported by the absence of apoptotic features observed in histochemical staining (Fig. 2C) and cleaved PARP expression in Western blot analysis (Fig. 2D and Supplementary Fig. 5B). However, Cur treatment resulted in very significant ($p < 0.01$) apoptotic activity (9.33 ± 0.4%) in MCF10A as compared to untreated control (1.73 ± 0.54%) as shown in Fig. 2Aii. Besides displaying chromatin condensation and cell shrinkage, nuclear chromatin fragmentation and apoptotic bodies formation were detected in Cur-treated MCF10A as shown in Fig. 2C. Moreover, Cur treatment resulted in cleavage of PARP in MCF10A as shown in Fig. 2D. Oppositely, Dox treatment did not cause significant effects on apoptotic studies.

In summary, administration of ZT augmented the TRAIL-induced apoptosis in human breast adenocarcinoma cells MDA-MB-231 at 24 h post-treatment, but this treatment was unharmed towards human breast epithelial cells MCF10A.

4. Discussion

TRAIL is an attractive candidate for anticancer therapy which is famously known for its preferentially killing of cancerous cells while sparing most normal cells (Ashkenazi et al., 1999; Wang, 2008). Although TRAIL was well-tolerated among cancer patients, TRAIL-based therapy exhibited no improved clinical outcomes (Soria et al., 2010, 2011). This is attributed by the development of various cancer defensive mechanisms towards TRAIL (Lemke et al., 2014; Wang and El-Deiry, 2003). A growing body of evidence supports the feasibility of combinational therapy in targeting TRAIL-induced apoptotic pathways (De Miguel et al., 2016; Frew et al., 2008). In this study, we explored the anticancer potential of Zeb and TSA in augmenting the TRAIL therapeutic effects and achieving maximal demise of human breast adenocarcinoma cells.

As aforementioned, many cancers develop resistance to TRAIL therapy (Zhang and Fang, 2004). Our results supported this notion whereby nominal reduction of cell viability was induced by TRAIL alone treatment towards MDA-MB-231. Oppositely, sensitization of Zeb and TSA greatly

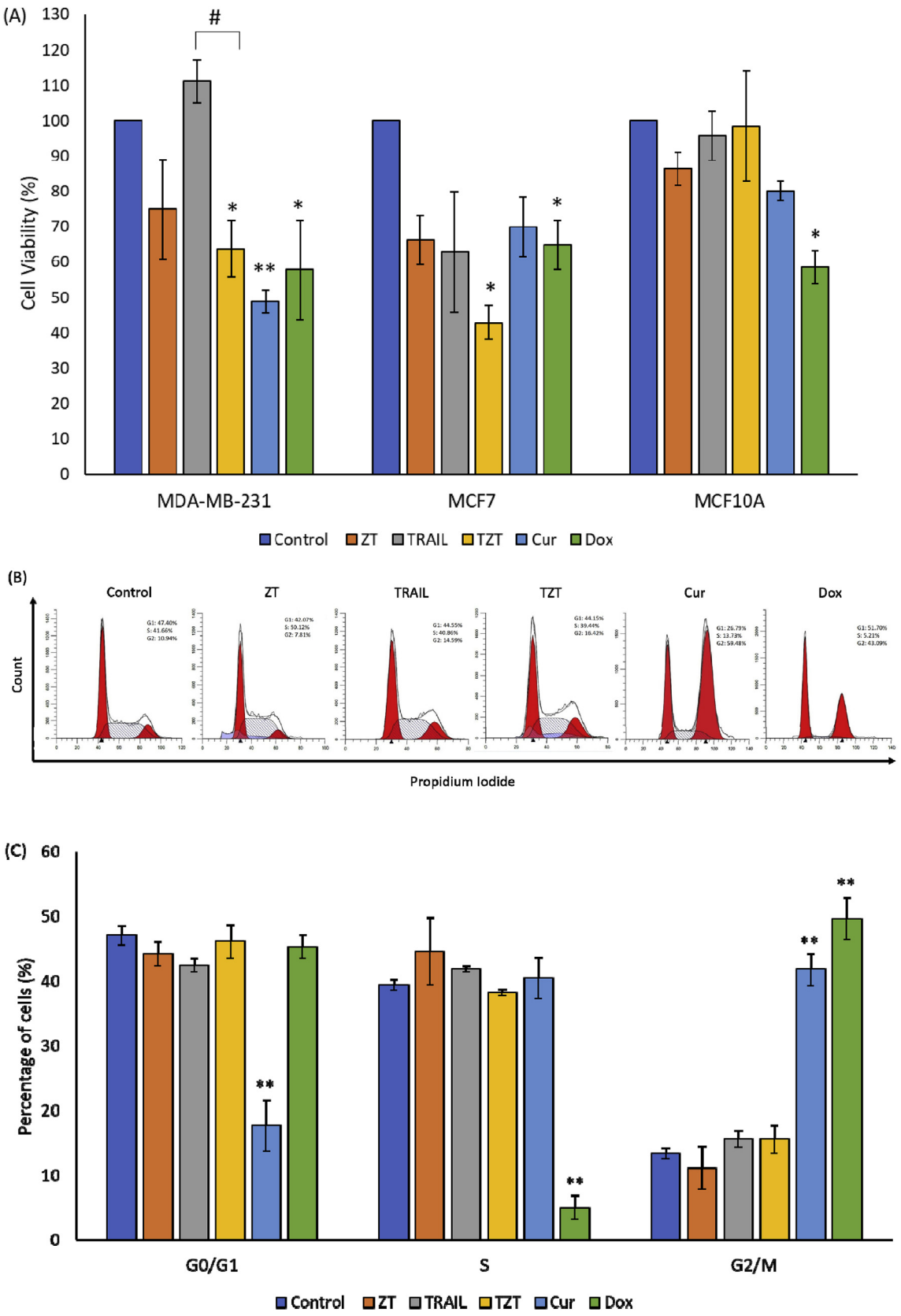
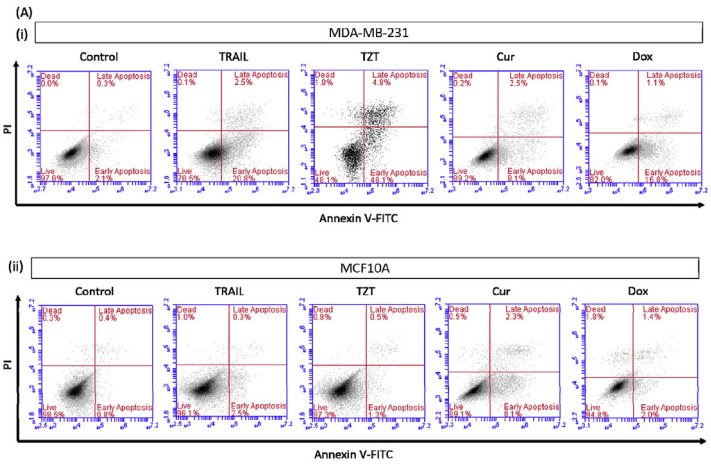
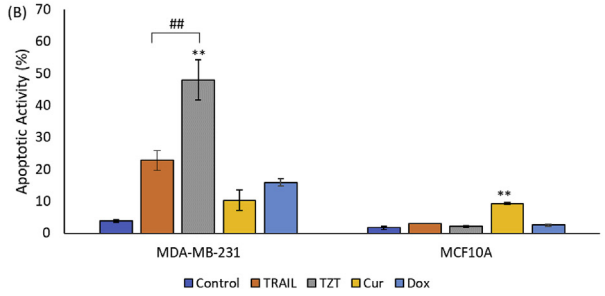
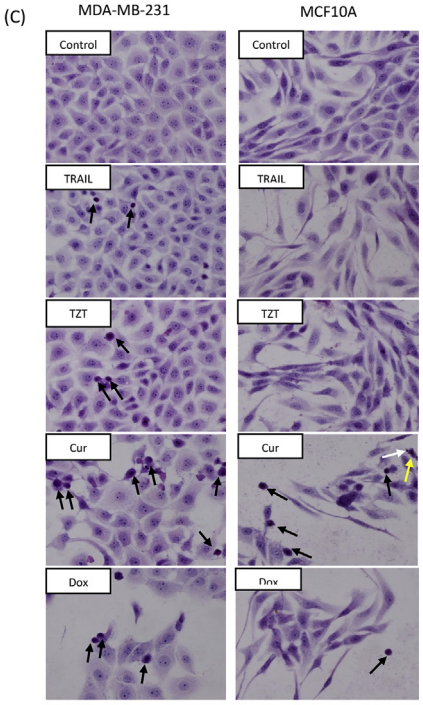


Fig. 1. Effects of Zeb and TSA as sensitizers to augment the TRAIL-induced apoptosis in human breast adenocarcinoma cells MDA-MB-231, MCF7 and breast epithelial cells MCF10A. (A) TZT treatment significantly reduced the cell viability of MDA-MB-231 but not MCF7 and MCF10A as compared to TRAIL treatment alone. (B) Representative flow cytometry data for cell cycle analysis in MDA-MB-231 in which the percentage of cells (%) arrested at each cell cycle phase is presented in (C) . (C) No changes in the cell cycle of the TRAIL and TZT treated MDA-MB-231. The data represented means ± SEM of triplicates in three independent samples. The significant difference was determined by *$p < 0.05$ and **$p < 0.01$ vs. control; #$p < 0.05$ vs. TRAIL treatment.







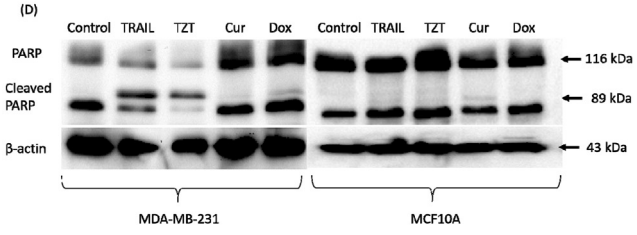


Fig. 2. Apoptotic analysis of human breast adenocarcinoma cells MDA-MB-231 and breast epithelial cells MCF10A under TRAIL, TZT, Cur and Dox treatments. (A) Representative flow cytometry data showed that TZT-treated MDA-MB-231 induced higher apoptotic activity as compared to TRAIL treatment while no apoptotic activities were found in MCF10A under TRAIL and TZT treatment. The apoptotic activity (%) of the cells is presented in (B). (B) Apoptotic activities of MDA-MB-231 and MCF10A under the treatments. The apoptotic activities were taken into account based on the cells undergo early apoptosis. (C) Apoptotic morphologies such as nuclear chromatin condensation and cell shrinkage (Black arrow); apoptotic bodies formation (White arrow); chromatin fragmentation (Yellow arrow) were evident in MDA-MB-231 but minimally observed in MCF10A under treatments. (D) TRAIL and TZT-treated MDA-MB-231 caused cleavage of PARP but not in MCF10A in Western blot analysis. The data represented means ± SEM of triplicates in two independent samples. The significant difference was determined by $^{**}p < 0.01$ vs. control; $\#\#p < 0.01$ vs. TRAIL treatment. The original uncropped blot images can be viewed in Supplementary Fig. 5.

decreased the cell viability of MDA-MB-231 towards TRAIL treatment. This sensitization effects can be related to the negative regulation of cell survival signalling and the reversal of TRAIL resistance mechanisms. For example, the sensitization had downregulated the cell survival signalling such as insulin growth factor-1 receptor (IGF-1R) signalling (Karasic et al., 2010) and phosphatidylinositol 3-kinase (PI3K) signalling (Wang et al., 2002; Xu et al., 2010) which eventually ceased the cell proliferation in cancerous cells. Besides, recent findings demonstrated the importance of sensitizers in reversing TRAIL resistance through the upregulation of tumour suppressor PTEN which is responsible for cell proliferation control (Xu et al., 2010).

Apart from the dysregulated cell viability, cancerous cells have uncontrolled cell division (Malumbres and Barbacid, 2009). We ought to explore whether the TRAIL and TZT can cause cell cycle arrest in human breast adenocarcinoma cells. Consistent with previous research, our results indicated no cell cycle arrest was induced in TRAIL- and TZT-treated MDA-MB-231 (Lin et al., 2011). With this, TRAIL was suggested to potentiate anticancer properties by other mechanisms, for instance, the induction of cell death mechanisms.

Although not causing a reduction in cell viability, TRAIL treatment elevated apoptotic activities in MDA-MB-231 via flow cytometry apoptotic analysis. This discrepancy can be explained by the interference of metabolic viability based on MTT assay when assessing cell viability. It is speculated that TRAIL could have protected the cells against mitochondria injury, increased the activity of mitochondrial succinate dehydrogenase and increased the production of formazan. Besides, TRAIL could also have interacted directly with MTT and caused the formazan formation. These activities could increase the formazan formation, thus overestimating the cell viability of the treated cells (Wang et al., 2010; Rai et al., 2018). Due to these limitations, flow cytometry apoptotic studies were conducted to confirm the apoptosis regulation under the TRAIL and TZT treatments towards MDA-MB-231. TRAIL initiates the cell death mechanisms through the extrinsic and intrinsic pathways. These apoptotic pathways trigger a series of caspase activities and cause the hallmark of apoptosis- cleavage of PARP proteins (Chaitanya et al., 2010). In TRAIL-resistant cancerous cells, these apoptotic pathways were highly governed by various anti-apoptotic proteins (De Miguel et al., 2016; Lemke et al., 2014). Our results proved the enhanced apoptotic activities by TZT treatment in MDA-MB-231 as compared to TRAIL alone treatment. The presence of two bands of the cleaved PARP remains unknown of its mechanisms. It could be due to the non-specific binding. However, it is evident that for the cells treated with TRAIL and TZT, the 89 kDa of the cleaved PARP was present, which is the most important research findings of this experiment. Besides, the apoptotic features such as nuclear chromatin condensation and cell shrinkage were observed through the histochemical staining in TRAIL- and TZT-treated MDA-MB-231. This agrees with other studies in which Zeb and TSA respectively augmented the TRAIL-induced apoptosis in cancerous cells through elevation of fucosylation level of death receptors (Moriwaki et al., 2010) and downregulation of anti-apoptotic proteins Bcl-2 (Billam et al., 2010; Park et al., 2009, 2012). Furthermore, other studies illustrated the additive effects by various sensitizers augmented TRAIL-induced apoptosis through the downregulation of cFLIP (Carlisi et al., 2009; Manouchehri et al., 2018), the reversal of DR internalizations (Moriwaki et al., 2010; Twomey et al., 2015) and the decreased expression of anti-apoptotic proteins (Hari et al., 2015; Park et al., 2014). Compared to TZT treatment, Cur and Dox treatments induced lesser apoptotic activities and PARP cleavage expression in MDA-MB-231. These results suggested that the Cur and Dox treatments reduced the cell viability through the modulation of cell cycle arrest, which is in accordance with the results obtained. This could be related to previous studies in which both Dox and Cur treatments induced G2/M cell cycle arrest while decreasing the cell viability of cancerous cells (Ramachandran and You, 1999; Meiyanto et al., 2011; Sun et al., 2012).

Besides illustrating the killing effects of TRAIL towards human breast adenocarcinoma cells, our findings were consistent with the concept that TRAIL is unharmed towards normal cell lines (Ashkenazi et al., 1999). Not only TRAIL treatment, TZT treatment exhibited very minimal reduction in cell viability and apoptotic activities in human breast epithelial cells. With this, it is suggested that TZT treatment is safe towards human breast epithelial cells. Compared to TZT, Cur induced apoptotic activities and cleavage of PARP in MCF10A. Besides decreasing cell viability, the apoptotic activity is postulated by the downregulation of CDK inhibitor p21 and upregulation of pro-apoptotic protein Bax in MCF10A treated with Cur which is related to previous studies (Ramachandran and You, 1999; Ramachandran et al., 2005). Although reducing cell viability of Dox-treated MCF10A, the apoptotic activity was rather minute. This could be related to a finding that normal cells were less responsive as compared to the cancer cells when both were treated with the same concentration of Dox (Stallard et al., 1990). This can be postulated that Dox only reduced cell viability and caused cell cycle arrest but not inducing apoptosis in this study.

In summary, Zeb and TSA collectively augmented the TRAIL effects towards human breast adenocarcinoma cells through the induction of apoptotic activities. Moreover, this therapeutic strategy caused no harm to the human breast epithelial cells. Further harnessing the molecular mechanisms and understanding the TRAIL resistance drivers, this therapeutic strategy can improve the clinical outcome among breast cancer patients in the future.

Declarations

Author contribution statement

Wei Yang Kong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zong-Yang Yee, Chun-Wai Mai, Chee-Mun Fang: Contributed reagents, materials, analysis tools or data. Syahril Abdullah: Analyzed and interpreted the data; Wrote the paper.

Siew Ching Ngai: Conceived and designed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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