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Cytotoxicity, Regulation of Apoptotic and Anti-apoptotic Gene Expression by IL-27 in MCF-7 and MDA-MB-231 Breast Cancer Cell Lines

(Sitotoksisiti, Pengawalaturan Pengekspresan Gen Apoptotik dan Antiapoptotik oleh IL-27 dalam Sel Selanjar Kanser Payudara MCF-7 dan MDA-MB-231)

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ABSTRACT

Breast cancer is one of the commonest cancers among women. Conventional therapies cause adverse side effects in patients. Cytokine immunotherapy such as interleukin-27 (IL-27) has been sought as an alternative cancer treatment in recent years. IL-27 has been shown to improve anticancer immunity and anti-angiogenesis in cancers, however, its effect on apoptotic and anti-apoptotic gene expression especially in breast cancers is yet to be explored. Cytotoxicity of IL-27 in non-cancerous (184b5) and cancerous (MCF-7 and MDA-MB-231) breast cell lines was first determined for 24-72 h in this study. The results indicated that IL-27 treatment did not retard 184b5 cell growth, however, did inhibit MCF-7 (48 h) and MDA-MB-231 (72 h) cell growth with IC_{50} at 442 and 457 ng/ml, respectively. Apoptotic (TRAIL, FADD, FAS, caspase-3 and caspase-8) and anti-apoptotic (BCL-2, AKT, and COX-2) genes were then amplified from untreated (control) and treated breast cancer cells and studied. TRAIL, caspase-3, caspase-8 gene expression was significantly ($p < 0.05$) upregulated in treated MCF-7 (442 ng/ml) and MDA-MB-231 (457 ng/ml) cells. Expression of FADD and FAS genes was not detected in both control and treated MCF-7 and MDA-MB-231 cells. COX-2 gene was also not expressed by MCF-7 cells, but reduced significantly ($p < 0.05$) in treated MDA-MB-231 cells. In MDA-MB-231 cells, IL-27 treatment seemed to slightly enhance the expression of AKT and BCL-2 genes which, on the other hand, was downregulated in treated MCF-7 cells. Conclusively, IL-27 is able to inhibit breast cancer cell growth and regulate apoptotic and anti-apoptotic gene expression in breast cancer cells.

Keywords: IL-27; cytokine immunotherapy; triple negative breast cancer; invasive ductal breast cancer

ABSTRAK

Kanser payu dara adalah antara kanser yang paling biasa dihidapi oleh wanita. Rawatan konvensional menyebabkan kesan sampingan teruk dalam pesakit. Imunoterapi sitokin seperti interleukin-27 (IL-27) telah digunakan sebagai rawatan alternatif sejak kebelakangan ini. IL-27 telah terbukti dapat menambah baik imuniti antikanser dan aktiviti anti-angiogenesis dalam kanser, namun kesannya pada pengekspresan gen apoptotik and anti-apoptotik terutamanya pada kanser payu dara masih belum diterokai. Dalam kajian ini, kesan sitotoksisiti IL-27 pada sel selanjar bukan kanser (184b5) dan kanser (MCF-7 dan MDA-MB-231) ditentukan pada 24-72 jam dalam kajian ini. Dapatan kajian menunjukkan rawatan IL-27 tidak menjejaskan pertumbuhan sel 184b5, namun menghalang pertumbuhan sel MCF-7 (48 jam) and MDA-MB-231 (72 jam) dengan nilai IC_{50} ialah 442 and 457 ng/ml, masing-masing. Gen apoptotik (TRAIL, FADD, FAS, caspase-3 dan caspase-8) and anti-apoptotik (BCL-2, AKT, dan COX-2) kemudiannya diamplifikasikan daripada sel kanser payu dara kawalan dan terawat serta dikaji. Pengekspresan gen TRAIL, caspase-3 dan caspase-8 telah meningkat secara signifikan ($p < 0.05$) dalam sel MCF-7 (442 ng/ml) and MDA-MB-231 (457 ng/ml) yang dirawat. Pengekspresan gen FADD dan FAS gagal dikesan dalam sel MCF-7 dan MDA-MB-231, mahupun sel kawalan atau terawat. Gen COX-2 tidak diekspreskan oleh sel MCF-7 tetapi pengekspresannya telah menurun secara signifikan ($p < 0.05$) dalam sel MDA-MB-231 yang dirawat. Dalam sel MDA-MB-231, rawatan IL-27 meningkatkan pengekspresan gen AKT dan BCL-2, namun pengekspresan kedua-dua gen tersebut telah menurun dalam sel MCF-7 yang dirawat. Kesimpulannya, IL-27 dapat menghalang pertumbuhan sel kanser payu dara dan mengawal atur pengekspresan gen apoptotik and anti-apoptotik dalam sel kanser payu dara.

Kata kunci: IL-27; imunoterapi sitokin; kanser payu dara negatif-ganda tiga; kanser duktus payu dara invasif

INTRODUCTION

Breast cancer accounts for 32.1% death among cancer patients in Malaysia (Azizah et al. 2016). Among all breast cancer cases, 10-20% of cases are triple negative breast cancer (TNBC) meanwhile 70-80% of breast cancers constitute of invasive ductal carcinoma (IDC) (Tommiska et al. 2007). Compared to IDC, TNBC is characterized by the absence of estrogen, progesterone and human epidermal growth receptors (Dent et al. 2007). It is, therefore, more aggressive and could readily metastasize to the other vital organs such as liver, brain and lung (Carey et al. 2010).

Conventional treatments such as chemotherapy and surgery have successfully increased breast cancer patients' life span (Isakoff 2010), however, they, at the same time, cause tremendous adverse side effects in patients such as immunosuppression, congestive heart failure, leukemia (Pan et al. 2014), emotional breakdown and depression due to physical changes (Arroyo & Lopez 2010). Owing to the shortcomings of currently available treatments for breast cancers, Stagg & Allard (2013) suggested that cytokine immunotherapy could be eligible alternative for treating breast cancer patients with negligible or minimal adverse effects. Cytokines can inhibit progression of cancer cells by augmenting anticancer immunity meanwhile promoting production of anti-angiogenic proteins such as IFN-γ and monokines (Shimizu et al. 2006). Such encouraging effects have been proven in a few clinical trials using aldesleukin (Interleukin 2, IL-2), protein CSPG4 and NY-ESO-1 (Ademuyiwa et al. 2012; Wang et al. 2010; Roberti et al. 2011) on breast cancer patients. Although these cytokines possessed effective anti-breast cancer activities, some recipients still experienced side effects such as fever, low platelet and white blood cells counts (Rosenberg et al. 2011; Sharma et al. 2011). To help address the concern, the use of interleukin-27 (IL-27) that has been proven to prohibit colon cancer and myeloma growth was proposed (Ho et al. 2009). IL-27 treatment has triggered very minimal side effects in preclinical trials using rat models (Yoshimoto et al. 2015). IL-27 is a heterodimeric cytokine that is composed of two protein subunits, Epstein-Barr virus gen induced-3 (EBVI-3) and p28 (Pflanz et al. 2002). Zarghi & Arfaei (2011) demonstrated that IL-27 was able to undermine the expression of anti-apoptotic factors such as COX-2 and PGE-2 in cancer cells. Besides, IL-27 also upregulates the protein expression level of interferon gamma (IFN-γ) which in turn triggers the production of apoptotic proteins such as caspases (Ruiz-Ruiz, Muñoz-Pinedo & López-Rivas 2000) that induce programmed cancer cell death.

Despite the proven anti-angiogenic effects and enhancement of anticancer immunity by IL-27, explicit regulatory effects of IL-27 on the expression of apoptotic and anti-apoptotic genes particularly in breast cancers are barely available. In view of this, the current study aimed to investigate the cytotoxicity, regulation of apoptotic and anti-apoptotic gene expression by IL-27 in MCF-7 and MDA-MB-231 breast cancer cell lines. The results indicated that IL-27 was able to obstruct breast cancer cell growth and regulate the expression of apoptotic and anti-apoptotic genes, hence, a potential candidate for cytokine immunotherapy in treating breast cancers.

MATERIALS AND METHODS

CELLS AND IL-27

Breast cancer cell lines, MDA-MB-231 and MCF-7 and non-cancerous breast cell line, 184b5 were purchased from American Type Culture Collection (ATCC) and were maintained in MEM medium (Sigma-Alrich, St. Louis, USA) supplemented with 10% (v/v) fetal bovine serum (Sigma-Aldrich, St Louis, USA) and 50 µg/ml PenStrep antibiotics (Sigma-Aldrich, St Louis, USA). Cells were grown at 37°C in a 5% CO_2 incubator. Interleukin-27 (IL-27) (Sigma-Aldrich, St. Louis, USA) was prepared as 10 µg/ml stock with nuclease-free water and then serially diluted to 50-500 ng/ml with MEM medium.

CYTOTOXICITY OF IL-27 IN NON-CANCEROUS AND CANCEROUS BREAST CELL LINES

Cytotoxicity of IL-27 in 184b5, MDA-MB-231 and MCF-7 cells was determined by using MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] assay kit (CellTiter 96® AQueous One Solution Cell Proliferation Assay) (Sigma-Aldrich, St Louis, USA). Cells (2×10^5 cells/ml) were cultured on 96-well plates and incubated overnight at 37°C under 5% CO_2. Cells were then washed three times with PBS and 50-500 ng/ml of IL-27 (Canale et al. 2011) was added to the cells in triplicate. Non-treated cells were prepared as negative control. IL-27 treatment was carried out for 24, 48, and 72 h. At each designated time point, the plate was collected and media was removed. Cells were washed with PBS and 100 µl fresh MEM medium was added to the cells. About 20 µl of CellTiter 96® AQueous One Solution reagent was added into each well and the cells were further incubated for 3 h at 37°C under 5% CO_2. The plate was read at 570 nm. Dose-response curve was conducted by using the absorbance readings. The value of 50% inhibitory concentration (IC_{50}) of IL-27 was derived from the curve.

ISOLATION OF TOTAL RNA

To allow collection of cells at larger number, MDA-MB-231 and MCF-7 cells (3×10^5 cells/ml) were cultured on a 6-well plate at 37°C under 5% CO_2 until 80% confluency was achieved. MCF-7 and MDA-MB-231 cells were treated with IL-27 at IC_{50} in duplicate for 48 and 72 h, respectively. After the treatment, the total RNA was extracted from the samples by using TRIzol RNA reagent (Invitrogen, New York, USA) as described in Tan, Hassan & Yap (2016). The extracted total RNA (250 ng) was used to generate cDNA using M-MLV reverse transcriptase (Promega Inc, Madison, USA).

AMPLIFICATION OF APOPTOTIC AND ANTI-APOPTOTIC GENES

Polymerase chain reaction (PCR) was carried out using GoTaq Plus DNA polymerase kit (Lucigen, Middleton, USA) according to the manufacturer's instructions. Apoptotic genes such as *TRAIL, FADD, FAS, caspase-3* and *caspase-8* and anti-apoptotic genes such as *COX-2, BCL-2* and *AKT* were amplified from untreated and treated breast cancer

cells. Primers used in the PCR amplification are shown in Table 1. *GAPDH* served as a housekeeping gene in this study. Thermal cycling conditions were as follows: initial denaturation: 95°C for 120 s, 30 cycles of denaturation at 95°C for 60 s, annealing at 55°C for 60 s and extension at 72°C for 60 s, final extension at 72°C for 300 s and the products were finally stored at 4°C. The PCR products were mixed with 6X DNA loading dye (Cleaver Scientific, Warwickshire, UK) and analysed on 1% (w/v) agarose gel at 70 V for 45 min. The agarose gel was then observed under a UV transilluminator box (Bio-Rad, California, USA). Gel pictures were captured and intensity of PCR products was analyzed using Image J (http://imagej.nih.gov). The intensity was measured and expressed as relative expression level to that of *GADPH*.

STATISTICAL ANALYSIS

Experiments were carried out in duplicate or triplicate. Data were expressed as mean ± S.D. Data were also analyzed with GraphPad Prism version 7.0. Non-linear regression test was used to determine the IC_{50} of the IL-27 in cancerous breast cells. Unpaired T-test was used to compare the gene expression levels in non-treated and treated breast cancer cells.

RESULTS

CYTOTOXICITY OF IL-27 IN NON-CANCEROUS AND BREAST CANCER CELL LINES

IL-27 treatment for 24 h did not affect the growth of non-cancerous, 184b5 and cancerous breast cells, MCF-7 and MDA-MB-231 (Figure 1a). MCF-7 breast cancer cells exhibited 100% viability while 184b5 breast cells remained growing in the range of 80 to 100%. Although slightly affected at higher concentrations of IL-27, MDA-MB-231 cells was still able to maintain at least 90% viability.

Figure 1(b) shows the effects of IL-27 treatment on the viability of the cells for 48 h. The viability of 184b5 and MDA-MB-231 remained relatively stable and unchanged which cell viability was at least 80%. However, a much lower viability was observed in MCF-7 breast cancer cell line especially when treated with 500 ng/ml of IL-27. The cell viability was only retained at 10%. Based on the curve, IC_{50} of IL-27 in MCF-7 was determined at 442 ng/ml.

As IL-27 treatment progressed to 72 h, none of the MCF-7 cells survived the treatment while 184b5 cells still did not exhibit any signs of toxicity from the treatment (Figure 1C). For MDA-MB-231 cells treated with IL-27 at 200-500 ng/ml, cell viability declined gradually in a concentration-dependent pattern. The cell viability was less than 50% after being treated with IL-27 at 500 ng/ml for 72 h, hence the IC_{50} of 457 ng/ml.

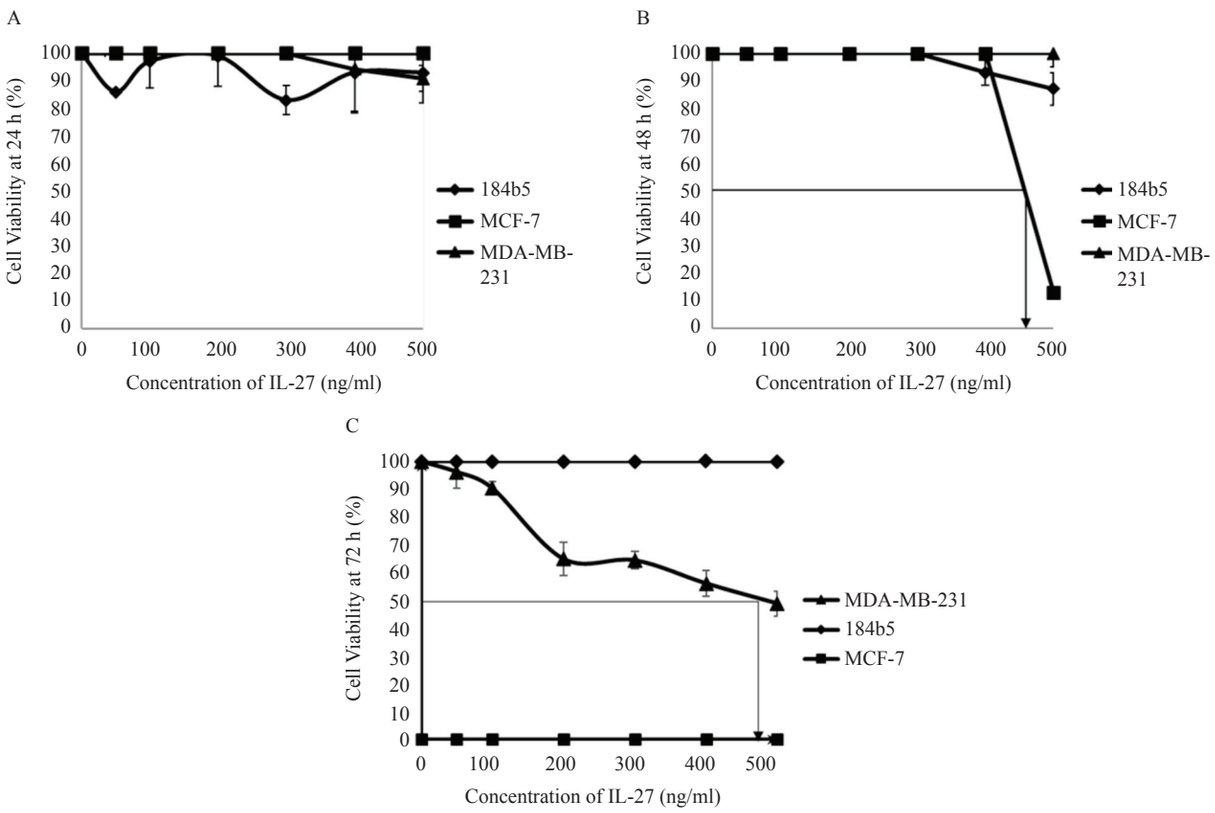

FIGURE 1. Cytotoxicity results of IL-27 treatment in non-cancerous and cancerous breast cell lines. 184b5, MCF-7 and MDA-MB-231 cells were treated with IL-27 for (A) 24 h; (B) 48 h; and (C) 72 h. Cell viability was determined using MTS assay kit.

MCF-7 (442 ng/ml) and MDA-MB-231 (457 ng/ml) cells were first treated with IL-27 at IC_{50} for 48 and 72 h, respectively. The effects of IL-27 treatment on the expression levels of apoptotic and anti-apoptotic genes were analyzed using PCR. As shown in Figure 2A and 2B, IL-27 treatment upregulated gene expression of *TRAIL*, *caspase-3* and *caspase-8* in both MCF-7 and MDA-MB-231 cell lines. Nevertheless, treatment with IL-27 was unable to upregulate the expression of *FAS* and *FADD* genes in the breast cancer cells.

Regulation of anti-apoptotic gene expression in MCF-7 and MDA-MB-231 breast cancer cells by IL-27 was also investigated in this study. In MCF-7 cells, expression of *AKT* and *BCL-2* genes decreased following IL-27 treatment (Figure 2C). However, the interleukin treatment seemed to slightly increase the expression of *AKT* and *BCL-2* genes in MDA-MB-231 cells (Figure 2D). *COX-2* gene was expressed minimally by untreated MDA-MB-231 cells. The gene expression was then suppressed by a 72-h treatment with IL-27 (Figure 2D). Unlike that observed in untreated and treated MDA-MB-231 cells, the *COX-2* gene expression was not detected in both untreated and treated MCF-7 cells.

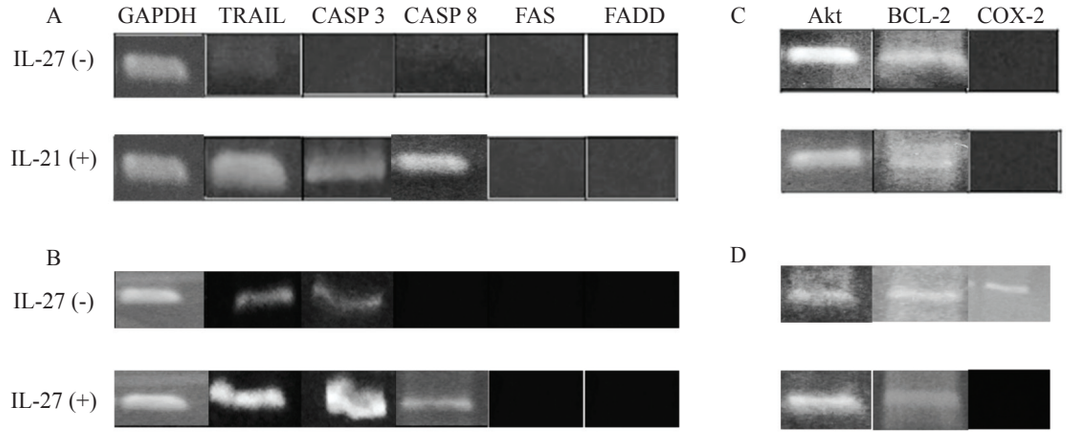

FIGURE 2. Expression of apoptotic and anti-apoptotic genes in non-treated and IL-27-treated breast cancer cells was analyzed on 1% (w/v) agarose gel. Panels: (A) expression of apoptotic genes in MCF-7; (B) expression of apoptotic genes in MDA-MB-231; (C) expression of anti-apoptotic genes in MCF-7; and (D) expression of anti-apoptotic genes in MDA-MB231. Symbols (+) and (-) indicate treated and non-treated samples, respectively.

Regulation of apoptotic and anti-apoptotic gene expression by IL-27 in breast cancer cells was further estimated by comparing the expression levels of those genes with the expression level of the housekeeping gene, *GADPH*. Based on Figure 3A, IL-27 treatment successfully upregulated the gene expression levels *of TRAIL* (0.7)*, caspase-3* (0.7) and *caspase-8* (0.6) significantly ($p < 0.05$) in treated MCF-7 cells compared to that of non-treated samples (undetectable). In MDA-MB-231 cells (Figure 3B), IL-27 treatment had managed to increase the relative gene expression levels of *caspase-3* (0.3 to 1.6) and *caspase-8* (undetectable to 1.8) significantly ($p < 0.05$). The relative gene expression level of *TRAIL* in IL-27-treated MDA-MB-231 cells also showed some level of increment (almost 1.2), however, the upregulation was not statistically significant enough ($p > 0.05$) when compared with that of non-treated cells.

As shown in Figure 3C, the relative gene expression level of *AKT* in MCF-7 cells declined from 0.7 to 0.6 after being treated with IL-27. The reduction was not statistically significant ($p > 0.05$). Similarly, the relative gene expression level of *BCL-2* in MCF-7 cells was also not significantly ($p > 0.05$) affected by IL-27 treatment. The relative gene expression level decreased from 0.4 to 0.3.

It is noteworthy that IL-27 treatment had, on the other hand, upregulated ($p < 0.05$) the relative expression levels of *AKT* and *BCL-2* in MDA-MB-231 cells (Figure 3D). Unlike the *AKT* and *BCL-2* genes, COX-2 gene expression was somewhat inhibited significantly ($p < 0.05$) by IL-27 in MDA-MB-231 cells.

DISCUSSION

Breast cancer is the one of most severe cancers affecting women globally (WHO 2014). According to statistics by the International Agency for Research on Cancer, Malaysia has 38.7% breast cancer incidence rate with 5410 new cases in 2012 alone (Yip, Pathy & Teo 2014). Norsaadah et al. (2005) explained that the increased risk of breast cancers among Malaysian women is mainly due to lifestyle and hereditary genetic factors. Chemotherapy, radiotherapy and mastectomy have long been used in breast cancer treatments (Wijayahadi et al. 2007). Although effective, these therapies nevertheless cause adverse side effects such as ovarian failure (Tiong et al. 2014) and neutropenia (Phua et al. 2012) in cancer patients. Therefore, Tan & Lota (2008) proposed immunotherapy as a novel therapeutic

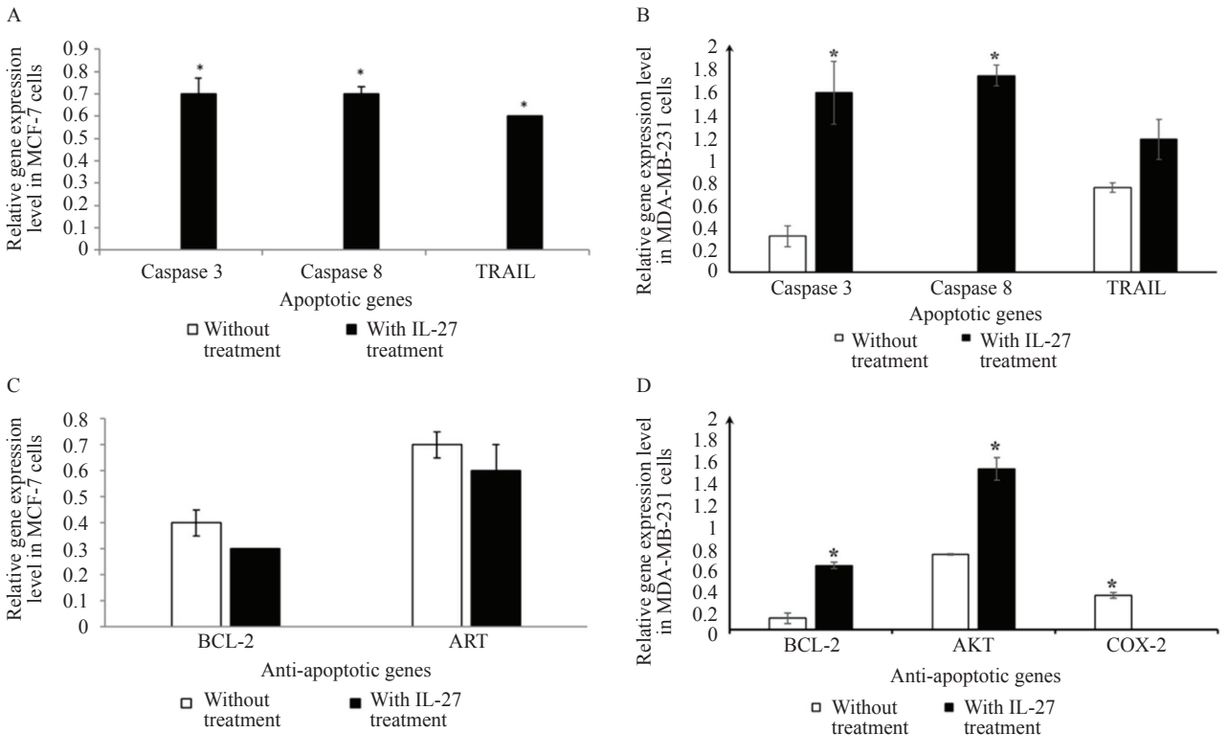

FIGURE 3. Relative gene expression levels of apoptotic genes in (A) MCF-7 cells; (B) MDA-MB-231 cells; and anti-apoptotic genes in (C) MCF-7 cells; (D) MDA-MB-231 cells. The relative gene expression levels were measured in relative to the basal gene expression level of *GADPH*. Symbol * represents the significance level ($p < 0.05$) in relative to the negative control.

TABLE 1. Primer sets used for the amplification of apoptotic and anti-apoptotic genes

Genes	Forward primers	Reverse primers	References
TRAIL	5' – GGATCATGGCTATGATGGAG – 3'	5' – GCGGCCGCAGTTAGCCAACTAA AAAGGC – 3'	Li et al. 2003
FAS	5' – GGAGGATTGCTCAACAACCAT – 3'	5' – CATTGTCATTCTTGATCTCATCT ATTT- 3'	Shen et al. 2002
FADD	5' – ATG GAC CCG TTC CTG GTG CT – 3'	5' – TCA GGA CGC TTC GGA GGT AGA – 3'	Yamada et al. 2014
Caspase-3	5' – TTAATAAAGGTATCCATGGAGAA CACT – 3'	5' – TTAGTGATAAAAATAGAGTTCTTT TGTGAG – 3'	Devarajan et al. 2002
Caspase-8	5' – AGAGTCTGTGCCCAAATCAAC – 3'	5' – GCTGCTTCTCTCTTTGCTGAA – 3'	Borhani et al. 2014
COX-2	5' – TCCAAATGAGATTGTGGGAAAAT TGCT – 3'	5'- AGATCATCTCTGCCTGAGTATCTT – 3'	Ochiai et al. 1999
AKT	5' – TCTATGGCGCTGAGAG – 3'	5' – CTTAATGTGCCCGTCCTT– 3'	Zhang et al. 2013
BCL-2	5' – ACT TGT GGC CCA GAT AGG CAC CCA G – 3'	5' – CGA CTT CGC CGA GAT GTC CAG CCA G – 3'	Dong et al. 1999
GADPH	5' – AAGGTGAAGGTCGGAGTCAAC – 3'	5' – GGGGTCATTGATGGCAACAATA – 3'	Borhani et al. 2014

strategy for breast cancer treatment. Immunotherapy stimulates specific anticancer immune cells that destroy and inhibit cancer cell growth without affecting the normal cells (Pflanz et al. 2002). In recent years, anticancer effects of cytokines have been widely studied as these signaling peptides are believed to be useful for immunotherapy especially in terms of enhancing anticancer immunity and, at the same time, activate immune responses that prohibit cancer cells from proliferating uncontrollably (Lee & Margolin 2011). As a member of cytokines with potent anticancer effects, IL-27 has been shown to inhibit anti-angiogenesis which in turns induces cancer cell death without causing inflammation and toxicity to normal cells (Lucas et al. 2003). For this reason, cytotoxicity, regulation of apoptotic and anti-apoptotic gene expression by IL-27 in breast cancers were studied using MDA-MB-231 and MCF-7 breast cancer cell lines in this study.

MCF-7 breast cancer cell line represented the major type (invasive ductal cancer, IDC) of breast cancers that is commonly found in breast cancer patients. IDC accounts for almost 80% of breast cancer incidences (Martinez & Azzopardi 1979). MDA-MB-231, on the other hand,

represented the aggressive, metastatic breast cancer that is responsible for approximately 20% of breast cancer cases (Tommiska et al. 2007).

Since there were no empirical data on cytotoxicity of IL-27 in non-cancerous and cancerous breast cell lines, the cytotoxicity assay was first performed at the concentration range of 50-500 ng/ml in order to determine the IC_{50} of IL-27 in both non-cancerous and cancerous breast cell lines. The concentration range was chosen according to Canale et al. (2011) that showed any IL-27 concentrations below 100 ng/ml did not significantly inhibit the growth of leukemic cells in acute B-lymphoma for 24 to 48 h of treatment. Even with increasing concentrations, anti-leukemic effects of IL-27 could only be observed at 200 ng/ml after 72 h of treatment. In this light, the concentration range of IL-27 was set at 50-500 ng/ml while IL-27 treatment was performed from 24-72 h in this study. The results showed that IL-27 treatment did not exert adverse toxic effects on non-cancerous breast cells, 184b5 but managed to inhibit more than 50% of MCF-7 and MDA-MB-231 breast cancer cell growth after 48- and 72-h treatments, respectively. This finding is in line with Dong et al. (2016) in which IL-27 was shown to be able to exert significant cytotoxicity to A549 lung carcinoma cells after 48 h of treatment. Besides, the cytotoxicity of IL-27 in both cancer cell lines also increased in a concentration-dependent manner. This observation is similar to that reported by Cocco et al. (2010). Compared to MCF-7 cells, IL-27 required longer duration in triggering MDA-MB-231 cell death. This is most likely because MDA-MB-231 is highly aggressive and resistant to treatment due to its triple-negative characteristics (Di Carlo et al. 2014). It was found that highly anticancer-resistant cancers would require treatment duration as long as 120 h for IL-27 to exert it cytotoxic effects on the cancer cells.

Chiba et al. (2013) demonstrated that IL-27 treatment was able to inhibit melanoma growth by regulating TRAIL pathway. Upregulation of *TRAIL* gene in both MCF-7 and MDA-MB-231 cells by IL-27 in this study correlates well with findings in that report. By enhancing TRAIL expression in cancer cells, apoptosis occurs and results in cancer cell death through activation of caspases such as caspase-3 and -8 (Wang & El Deiry 2003). *Caspase-3* and *-8* gene expression levels in breast cancer cells were found increased by IL-27 treatment in this study. This result supports findings by Tang, Lahti and Kidd (2000) in which caspase-3 activation was an important key for the generation of functional caspase-8 in MCF-7 cells. In view of this, together with upregulation of TRAIL gene expression, it is probable that IL-27 induced cell death in MCF-7 and MDA-MB-231 via TRAIL/caspase 3/caspase 8 pathway.

Expression of *FAS* and *FADD* genes was not affected by IL-27 treatment in both of the breast cancer cell lines. Previous findings showed that MCF-7 and MDA-MB-231 breast cancer cells were less responsive to *FAS* and *FADD* gene expression even when treated with anti-cancer agents such as IFN-γ at 10 times higher concentration (Keane et al. 1996; Mullauer et al. 2000). Furthermore, Day, Huang & Safa (2008) reported the presence of FAS and FADD inhibitor, namely cellular-Flic inhibitory protein (c-FLIP) in MCF-7 cells, hence significantly low FAS and FADD gene expression in the breast cancer cells. However, this speculation requires further empirical authentication.

IL-27 treatment slightly lowered *AKT* and *BCL-2* gene expression in MCF-7 cells in this study. Reduction of *AKT* and *BCL-2* gene expression had been shown to speed up melanoma cell death through anti-angiogenesis (Cocco et al. 2010) and apoptosis in cancer cells by release of cytochrome-c (Cory, Huang & Adams 2003). Youle & Strasser (2008) also found that it is important for breast cancer cells to maintain high BCL-2 level in order to sustain their growth. Therefore, a slight reduction of BCL-2 level could wreak dramatic change such as cell death in breast cancer cells as seen in this study.

Unlike MCF-7 breast cancer cells, the gene expression levels of *AKT* and *BCL-2* were somehow upregulated by IL-27 in MDA-MB-231 cells. A similar observation was reported by Chen et al. (2001). In the report, it was clearly demonstrated that even if the anti-apoptotic genes were upregulated in cancer cells, the relatively high expression level of *TRAIL* gene was able to block their anti-apoptotic activities hence cancer cell death. Besides, although often regarded as an anti-apoptotic gene, BCL-2 is also responsible for the activation of certain apoptotic molecules such as BAX and BAD, therefore leading to cancer cell death (Delbridge et al. 2016) as observed in IL-27 treated MDA-MB-231 cells in this study. It is noteworthy that further laboratory investigations are required to authenticate these speculations rigorously.

Half et al. (2002) indicated that *COX-2* gene expression was commonly knocked down in MCF-7 but not in MDA-MB-231 cells. The *COX-2* gene expression was downregulated by IL-27 treatment in MDA-MB-231 cells. The inhibition could possibly occur STAT-1 pathway that eventually causes cell death (Tsatsanis et al. 2006).

CONCLUSION

Although this study presents preliminary evidence on cytotoxicity of IL-27 in non-cancerous and cancerous breast cell lines, it offers vital information on the potential use of IL-27 in treating breast cancers without causing much side effects to patients. Early evidence describing how IL-27 regulates apoptotic and anti-apoptotic gene expression in breast cancer cells, on the other hand, provide important insight for further laboratory investigations so IL-27 can be used more specifically and precisely as a potential anti-breast cancer cytokine. Conclusively, IL-27 holds promise as a potential interleukin candidate in cytokine immunotherapy for breast cancers.

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REFERENCES

Ademuyiwa, F.O., Bshara, W., Attwood, K., Morrison, C., Edge, S.B., Ambrosone, C.B., O'connor, T.L., Levine, E.G., Miliotto, A., Ritter, E., Ritter, G., Gnjatic, S. & Odunsi, K. 2012. Ny-Eso-1 cancer testis antigen demonstrates high immunogenicity in triple-negative breast cancer. PLoS ONE; 7(6): e38783.

Azizah, A.M., Nor Saleha, I.T., Noor Hashimah, A., Asmah, Z.A. & Mastulu, W. 2016. Malaysian National Cancer Registry Report 2007-2011 Malaysia Cancer Stastistics, Data and Figure. Putrajaya, Malaysia National Cancer Institute.

Arroyo, J.M.G. & López, M.L.D. 2011. Psychological problems derived from mastectomy: a qualitative study. Int J Surg Oncol; 2011(2011): http://dx.doi.org/10.1155/2011/132461.

Borhani, N., Manoochehri, M., Saleh Gargari, S., Ghaffari Novin, M., Mansouri, A. & Omrani, M.D. 2014. Decreased expression of proapoptotic genes caspase-8 and Bcl-2-associated agonist of cell death (Bad) in ovarian cancer. *Clin Ovarian Cancer Other Gynecol Cancers* 7: 18-23.

Canale, S., Cocco, C., Frasson, C., Seganfreddo, E., Di Carlo, E., Ognio, E., Sorrentino, C., Ribatti, D., Zorzoli, A., Basso, G., Dufour, C. & Airoldi, I. 2011. Interleukin-27 inhibits pediatric B-acute lymphoblastic leukemia cell spreading in a preclinical model. *Leukemia* 25(12): 1815-24.

Carey, L., Winer, E., Viale, G., Cameron, D. & Gianni, L. 2010. Triple-negative breast cancer: disease entity or title of convenience? *Nat Rev Clin Oncol* 7(12): 683-692.

Chen, Q., Gong, B., Mahmoud-Ahmed, A.S., Zhou, A., Hsi, E.D., Hussein, M. & Almasan, A. 2001. Apo2l//Trail and Bcl-2-related proteins regulate type-I interferon-induced apoptosis in multiple myeloma. *Blood* 98: 2183-2192.

Chiba, Y., Mizoguchi, I., Mitobe, K., Mitobe, K., Higuchi, K., Nagai, H., Nishigori, C., Mizuguchi, J. & Yoshimoto, T. 2013. IL-27 enhances the expression of TRAIL and TLR3 in human melanomas and inhibits their tumor growth in cooperation with a TLR3 agonist poly(I:C) partly in a TRAIL dependent manner. *Plos ONE* 8: e76159.

Cocco, C., Giuliani, N., Di Carlo, E., Ognio, E., Storti, P., Abeltino, M., Sorrentino, C., Ponzoni, M., Ribatti, D. & Airoldi, I. 2010. Interleukin-27 Acts as Multifunctional Antitumor Agent in Multiple Myeloma. *Clin Cancer Res* 16(6): 4188-4197.

Cory, S., Huang, D.C.S. & Adams, J.M. 2003. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene* 22: 8590-8607.

Day, T.W., Huang, S. & Safa, A.R. 2008. C-FLIP knowkdown induces ligand-independent DR5-, FADD-, caspase-8-, and caspase-9-dependent apoptosis in breast cancer cells. *Biochem Pharmacol* 76: 1694-1704.

Delbridge, A.R.D., Grabow, S., Strasser, A. & Vaux, D.L. 2016. Thirty years of Bcl-2: translating cell death discoveries into novel cancer therapies. *Nat Rev Cancer* 6(2): 99-109.

Dent, R., Trudeau, M., Pritchard, K.I., Hanna, W.M., Kahn, H.K., Sawka, C.A., Lickle, L.A., Rawlinson, E., Sun, P. & Narod, S.A. 2007. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 13(15): 4429-4434.

Devarajan, E.S.A., Chen, J.S., Krishnamurthy, R.R., Aggarwal, N., Brun, A.M., Sapino, A., Zhang, F., Sharma, D., Yang, X.H., Tora, A.D. & Mehta, K. 2002. Downregulation of caspase-3 in breast cancer: a possible mechanism for chemoresistance. *Oncogene* 21: 8843-8851.

Di Carlo, E., Sorrentino, C., Zorzoli, A., Di Meo, S., Tupone, M.G., Ognio, E., Mincione, G. & Airoldi, I. 2014. The antitumor potential of interleukin-27 in prostate cancer. *Oncotarget* 5(21): 10332-10341.

Dong, L., Wang, W., Wang, F., Stoner, M., Reed, J.C., Harigai, M., Samudio, I., Kladde, M.P., Vyhlidal, C. & Safe, S. 1999. Mechanisms of transcriptional activation of Bcl-2 gene expression by 17β-estradiol in breast cancer cells. *J Biol Chem* 274(45): 32099-32107.

Dong, Z., Tai, W., Lei, W.L., Wang, Y., Li, Z.K. & Zhang, T. 2016. IL-27 inhibits the TGF-β1-induced epithelial-mesenchymal transition in alveolar epithelial cells. *BMC Cell Biol* 17(7): https://doi.org/10.1186/s12860-016-0084-x.

Half, E., Tang, X.M., Gwyn, K., Sahin, A., Wathen, K. & Sinicrope, F.A. 2002. Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma *in situ*. *Cancer Res* 62(6): 1676-1681.

Ho, M.Y., Leu, S.J., Sun, G.H., Tao, M.H., Tang, S.J. & Sun, K.H. 2009. IL-27 directly restrains lung tumorigenicity by suppressing cyclooxygenase-2-mediated activities. *J Immunol* 183(10): 6217-6226.

Isakoff, S.J. 2010. A phase II trial of the parp inhibitor Veliparib (Abt888) and Temozolomide for metastatic breast cancer. *J Clin Oncol* 28(15 Suppl.): a1019.

Keane, M.M., Ettenberg, S.A., Lowrey, G.A., Russell, E.K. & Lipkowitz, S. 1996. Fas expression and function in normal and malignant breast cell lines. *Cancer Res* 56: 4791-4798.

Lee, S. & Margolin, K. 2011. Cytokines in cancer immunotherapy. *Cancers* 3: 3856-3893.

Li, J.H., Kirkiles-Smith, N.C., Mcniff, J.M. & Pober, J.S. 2003. Trail induces apoptosis and inflammatory gene expression in human endothelial cells. *The J Immunol* 171(3): 1526-1533.

Lucas, S., Ghilardi, N., Li, J. & De Sauvage, F.J. 2003. IL-27 regulates IL-12 responsiveness of naïve CD4+ T-cells through Stat1-dependent and -independent mechanisms. *PNAS* 100(25): 15047-15052.

Martinez, V. & Azzopardi, J.G. 1979. Invasive lobular carcinoma of the breast: incidence and variants. *Histopathol* 3(8): 467-488.

Müllauer, L., Mosberger, I., Grusch, M., Rudas, M. & Chott, A. 2000. Fas ligand is expressed in normal breast epithelial cells and is frequently up-regulated in breast cancer. The J Pathol; 190(1): 20-30.

Norsaadah, B., Rusli, B.N., Imran, A.K., Naing, I. & Winn, T. 2005. Risk factors of breast cancer in women in Kelantan, Malaysia & Singapore. *Med J Malaysia* 46(12): 698-705.

Ochiai, M., Oguri, T., Isobe, T., Ishioka, S. & Yamakido, M. 1999. Cyclooxygenase-2 (Cox-2) mRNA expression levels in normal lung tissues and non-small cell lung cancers. *Jap J Cancer Res* 90(12): 1338-1373.

Pan, K., Guan, X.X., Li, Y.Q., Zhao, J.J., Li, J.J., Qiu, H.J., Weng, D.S., Wang, Q.J., Liu, Q, Huang, L.X., He, J., Chen, S.P., Ke, M.L., Zeng, Y.X. & Xia, J.C. 2014. Clinical activity of adjuvant cytokine-induced killer cell immunotherapy in patients with post-mastectomy triple-negative breast cancer. *Clin Cancer Res* 20(11): 3003-3011.

Phua, C.E., Bustam, A.Z., Yusof, M.M., Saad, M., Yip, C.H., Taib, N.A., Ng, C.H. & The, Y.C. 2012. Risk of treatment related death and febrile neutropaenia with taxane-based adjuvant chemotherapy for breast cancer in a middle income country outside a clinical trial setting. *Asian Pac J Cancer Prev* 13(9): 4623-4626.

Pflanz, S., Timans, J.C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., Blumenschein, W.M., Mattson, J.D., Wagner, J.L., To, W., Zurawski, S., McClanahan, T.K., Gorman, D.M., Bazan, J.F., de Waal Malefyt, R., Rennick, D. & Kastelein, R.A. 2002. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells. *Immunity* 16: 779-790.

Roberti, M.P., Barrio, M.M., Bravo, A.I., Rocca, Y.S., Arriaga, J.M., Bianchini, M., Mordoh, J. & Levy, E.M. 2011. IL-15 and IL-2 increase Cetuximab-mediated cellular cytotoxicity against triple-negative breast cancer cell lines expressing Egfr. *Breast Cancer Res Treat* 130(2): 465-475.

Rosenberg, S.A., Yang, J.C., Sherry, R.M., Kammula, U.S., Hughes, M.S., Phan, G.Q., Citrin, D.E., Restifo, N.P., Robbins, P.F., Wunderlich, J.R., Morton, K.E., Laurencot, C.M., Steinberg, S.M., White, D.E. & Dudley, M.E. 2011. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res* 17(13): 4550-4557.

Ruiz-Ruiz, C., Muñoz-Pinedo, C. & López-Rivas, A. 2000. Interferon-gamma treatment elevates caspase-8 expression and sensitizes human breast tumor cells to a death receptor-induced mitochondria-operated apoptotic program. *Cancer Res* 60(20): 5673-80.

Sharma, P., Wagner, K., Wolchok, J.D. & Allison, J.P. 2011. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. *Nat Rev Cancer* 11(11): 805-812.

Shen, L., Liang, A.C.T., Lu, L., Au, W.Y., Kwong, Y.L., Liang, R.H.S. & Srivastava, G. 2002. Frequent deletion of Fas gene sequences encoding death and transmembrane domains in nasal natural killer/T-cell lymphoma. *Am J Pathol* 161(6): 2123-2131.

Stagg, J. & Allard, B. 2013. Immunotherapeutic approaches in triple-negative breast cancer: latest research and clinical prospects. *Ther Adv Med Oncol* 5(3): 169-181.

Shimizu, M., Shimamura, M., Owaki, T., Asakawa, M., Fujita, K., Kudo, M., Iwakura, Y., Takeda, Y., Luster, A.D., Mizuguchi, J. & Yoshimoto, T. 2006. Antiangiogenic and antitumor activities of IL-27. *J Immunol* 176(12): 7317-7324.

Tan, P.H. & Lota, A.S. 2008. Interaction of current cancer treatments and the immune system: implications for breast cancer therapeutics. *Exp Opin Pharmacother* 9: 2639-2660.

Tang, D., Lahti, J.M. & Kidd, V.J. 2000. Caspase-8 activation and bid cleavage contribute to MCF7 cellular execution in a caspase-3-dependent manner during staurosporine-mediated apoptosis. *J Biol Chem* 275(13): 9303-9307.

Tan, T.S., Syed Hassan, S. & Yap, W.B. 2016. Significant replication time-points of avian influenza A virus strain H5N1 in Madin-Darby Canine Kidney cells. *Malays J Med Health Sci* 14 (1): 17-21.

Tiong, V., Rozita, A.M., Taib, N.A., Yip, C.H. & Ng, C.H. 2014. Incidence of Chemotherapy-Induced Ovarian Failure in Premenopausal Women Undergoing Chemotherapy for Breast Cancer. *World J Surg* 38(9): 2288-2296.

Tommiska, J., Bartkova, J., Heinonen, M., Hautala, L., Kilpivaara, O., Eerola, H., Aittomaki, K., Hofstetter, B., Lukas, J., Von Smitten, K., Blomqvist, C., Ristimaki, A., Heikkila, P., Bartek, J. & Nevanlinna, H. 2007. The DNA damage signaling kinase Atm is aberrantly reduced or lost in Brca1/Brca2-Deficient and Er/Pr/Erbb2-Triple-Negative Breast Cancer. *Oncogene* 27(17): 2501-2506.

Tsatsanis, C., Androulidaki, A., Venihaki, M. & Margioris, A.N. 2006. Signaling networks regulating cyclooxygenase-2. *Int J Biochem Cell Biol* 38(10): 1654-1661.

Wang, S. & El-Deiry, W.S. 2003. TRAIL and apoptosis induction by TNF-family death receptors. *Oncogene* 22(53): 8628-8633.

Wang, X., Osada, T., Wang, Y., Yu, L., Sakakura, K., Katayama, A., Mccarthy, J.B., Brufsky, A., Chivukula, M., Khoury, T., Hsu, D.S., Barry, W.T., Lyerly, H.K., Clay, T.M. & Ferrone, S. 2010. Cspg4 protein as a new target for the antibody-based immunotherapy of triple-negative breast cancer. *J Natl Cancer Inst* 102(19): 1496-1512.

Wijayahadi, N., Haron, M.R., Stanslas, J. & Yusuf, Z. 2007. Changes in cellular immunity during chemotherapy for primary breast cancer with anthracycline regimens. *J Chemother* 19(6): 716-23.

World Health Organization – Cancer Country Profiles, 2014.

Yamada, N., Noguchi, S., Kumazaki, M., Shinohara, H., Miki, K., Naoe, T. & Akao, Y. 2014. Epigenetic regulation of microRNA-128a expression contributes to the apoptosis-resistance of human T-cell leukaemia Jurkat cells by modulating expression of Fas-associated protein with death domain (FADD). *Biochim Biophys Acta* 1843(3): 590-602.

Yip, C.H., Pathy, N.B. & Teo, S.H. 2014. A Review of Breast Cancer Research in Malaysia. *Med J Malaysia* 69: 8-22.

Yoshimoto, T., Chiba, Y., Furusawa, J.I., Xu, M., Tsunoda, R., Higuchi, K. & Mizoguchi, I. 2015. Potential clinical application of interleukin-27 as an antitumor agent. *Cancer Sci* 106(9): 1103-1110.

Youle, R.J. & Strasser, A. 2008. The BCL-2 protein family: opposing activities that mediate cell death. *Nature* 9: 47-59.

Zarghi, A. & Arfaei, S. 2011. Selective COX-2 inhibitors: a review of their structure-activity relationships. *Iran J Pharm Res* 10(4): 655-683.

Zhang, J.U.N., Zhang, L.L., Shen, L.E.I., Xu, X.M. & Yu, H.G. 2013. Regulation of Akt gene expression by cisplatin. *Oncol Lett* 5(3): 756-760.

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