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Inhibition of Growth MCF-7 Breast Cancer Cell with Umbilical Cord Mesencymal Stem Cells (UCMSCs) Derived Secretome

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Abstract

Background: Breast cancer is the most common cancer affecting in women and is expected will be increase by 2040. Conventional therapy with chemotherapy as the most widely used therapy has severe side effects and can develop chemotherapy resistance. Currently, Mesenchymal stem cells (MSCs) derived secretome have become an alternative for cancer treatment because it contains various soluble factors, such as cytokines or growth factors, and extracellular vesicles. This study aims to evaluate the anticancer effect of secretome on MCF-7 cancer cells. Methods: MSCs were extracted from the umbilical cord and this supernatant was collected as secretome. The cytotoxic effect of secretome on MCF-7 was examined at concentrations 25-60% with MTT method and the optimum concentration (IC_{50}) was calculated. Result: Secretome showed significantly reduced proliferation and had cytotoxic effects on the MCF-7 cell line after 75 h treatment with IC_{50} 50%. Conclution: Secretome has anticancer effect and could significantly reduce cell viability and proliferation.These findings suggest that secretome is a promising candidate for breast cancer theraphy. Further studies are needed to evaluate the mechanism of action of secretome in inhibit the growth of breast cancer cell.

Keyword: Breast cancer, MCF-7, MTT assay, Secretome, UCMSCs.

Introduction

Breast cancer (BC) is a cancer attacking in women worldwide that remains a significant public health challenge (1,2). An estimated 1.7 million women have been diagnosed with BC and could elevate to 19.3 million and is expected to reach 28.4 million by 2040 (3,4). Breast cancer is characterized by the progression of abnormal cells in the inner lining of the mammary ducts or lobules, which can be caused by sequential mutations that alter the function of various cell types. Breast cancer has been classified based on the morphological arrangement of the cells, the degree of invasion, and the frequency of occurrence. These factors influence treatment strategies(5). At present, there are three a comprehensive BC treatment approaches: surgery, radiotherapy, and chemotherapy (6-8). However, chemotherapy as the most widely use have severe side effects and can develop chemoresistances (7).

One possible alternative innovative treatment is utilise of MSCs (9). MSCs play an important role in controlling of intercellular signaling cascades of the anti-inflammatory, anti-apoptotic, angiogenic, and anti-tumor. This is because MSCs secrete various soluble factors, such as cytokines or growth factors, and extracellular vesicles (9,10). Considering that, previous study has investigated the antitumor effects

of MSCs in melanoma cancer in vitro and in vivo (11), colon cancer (12) and triple negative breast cancer(13).

Despite from the above-mentioned studies, similar findings from other studies indicate the potent anti-tumor effects of MSC-derived exoxomes (14) and MSC derived extracellular vesicles (EV)(15). In this regard, we describe other type MSC derived called MSCs derived secretome. The secretome contains various molecules that can influence gene expression and intercellular communication. Furthermore, the secretome contributes to various physiological processes through paracrine mechanisms, thus showing potential as a therapeutic option for various diseases and demonstrating significant advantages as a cell-free therapy (16). Based on these data, we propose to study the anticancer effects of the secretome derived from MSCs on breast cancer cells.

Materials and Methods

Secretome collection

MSCs derived secretome were isolated from umbilical cord. Prior to isolation, patient screening is performed using serological tests, including HIV test, syphilis, cytomegalovirus, toxoplasma, rubella, and hepatitis viruses. Only non-reactive results are processed further.

Umbilical cord (UC) tissue samples were mechanically and enzymatically homogenized using collagenase. The tissue was cultured in 25 and 75 cm^2 flasks until 80-90% confluence in α-MEM medium supplemented with FBS, glutamax (Gibco), pens-strep, and sodium bicarbonate. The entire process was carried out in a biosafety cabinet under sterile conditions.

Secretome were isolated as previously described by Mirabdollahi et al with some modification(17). Secretome from UC was obtained at 4^{th} passage with 80% cell culture confluence. Removed the supernatant and take the cells then washed with phosphate buffer saline (PBS). Incubated the cells in α-MEM medium without FBS and antibiotics for 48 h. The medium was collected and centrifuge for 10 min at 2000 g and the supernatant was collected and stored at -80^0C until used.

Culture of MCF-7 breast cancer cells

MCF-7 cells were culture as our previous studies (18,19). Cells were cultured in RPMI 1640 supplemented with FBS, pen-strep and incubate at 37°C, 5% CO_2 incubator humidity. Exponentially growing cells (-80% confluency) were used for this study.

Cytotoxicity assays and cell morphology

We assesed the inhibitory concentration (IC_{50}) of secretome in concentrations 25-60% with MTT methods. Cells at a density of 2×10^4 cells/well were seeded then incubate for 24h. A total 100 µl of secretome wash added then incubated in three different time (24, 48 and 72h). Afterward, a total of 20 µl of MTT solution was added and incubated for 3h. To dissolve the formazan crystals,100 µl of dimethyl sulfoxide wass added and analyzed at ʎ 570 nm by an ELISA reader. The % of cell viability was calculated according to the formula:

$$\% \text{ cell viability} = ((Abs_{sample} - Abs_{control})/Abs_{control}) \times 100\%$$

Morphological changes treated and treated cells were assesed using inverted microscope at 100x magnification (19)plants have been extensively explore for their bioactive compound that is effective as anticancer drug candidates. Gnetum gnemon L. plant contains a bioactive compound that is beneficial for health and can be developed as an anticancer agent. The aim of this study was to investigate the potential of Gnetum gnemon L. seed extract as an antioxidant and anticancer in two cells line, MCF-7 and HeLa cells. Methods: The antioxidant evaluated through the DPPH (2,2-Diphenyl- 1-picrylhydrazyl.

Statistical analysis

The analysis was performed with MS Excel and SPSS version 25. Analysis of Vari-

ance (ANOVA) was used to analyze the data and further statistical tests such Tuckey's HSD post hoc test at α = 5% were applied. The IC_{50} value of the cytotoxic effect was analyzed using probit analysis.

Results and Discussion

Cytotoxicity assays and cell morphology

Cytotoxic of UCMSCs derived secretome were evaluated at different concentration of MCF-7 cells and the result after three replicates showed that the secretome could have cytotoxic effect on MCF-7 cells. The IC50 was determined as 50% (Table.1). UCMSCs derived secretome decreased the survival of the cells concentration-dependently. The survival rate (Table.1) decreased in the 25% secretome exposed cells compared to the control ($p<0.05$).

Table 1: Cytotoxicity assays against MCF-7 cancer cells

Secretome (%)	% cell viability	Cytotoxic activity (IC_{50},%) MCF-7(mean ± SD)
60	13,77 ± 1,62[a]	50,20 ± 6,64
50	24,35 ± 1,75[b]	
40	41,86 ± 9,82[a]	
30	49,80 ± 6,64[bc]	
25	62,47 ± 7,48[c]	
0	100 ± 0,00[d]	
Doxorubicin		

Data are expressed as mean ± standard deviation; different letters in the same column are significant differences at $p < 0,05$ (Tuckey's HSD post hoc test).

Optical microscope analysis of MCF-7 morphology after treated with secretome shows morphological changes in cell treated with 50% secretome for 72 h (. Figure.1d), as compared with untreated control cells (Figure.1a) in 100 x magnification.

To evaluate the impact of UCMSCs derived secretome on the behavior of breast cancer cells was investigated. Treatment of MCF-7 cells with secretome led to a reduction in cell proliferation. UCMSCs derived secretome significantly inhibited the viability of MCF-7 after treatment for 72 h (80.37% vs. 24 h treatment, $p=0.024(p<0.05)$), exhibited lower density and abundant cell debris formation (Figure.1e).

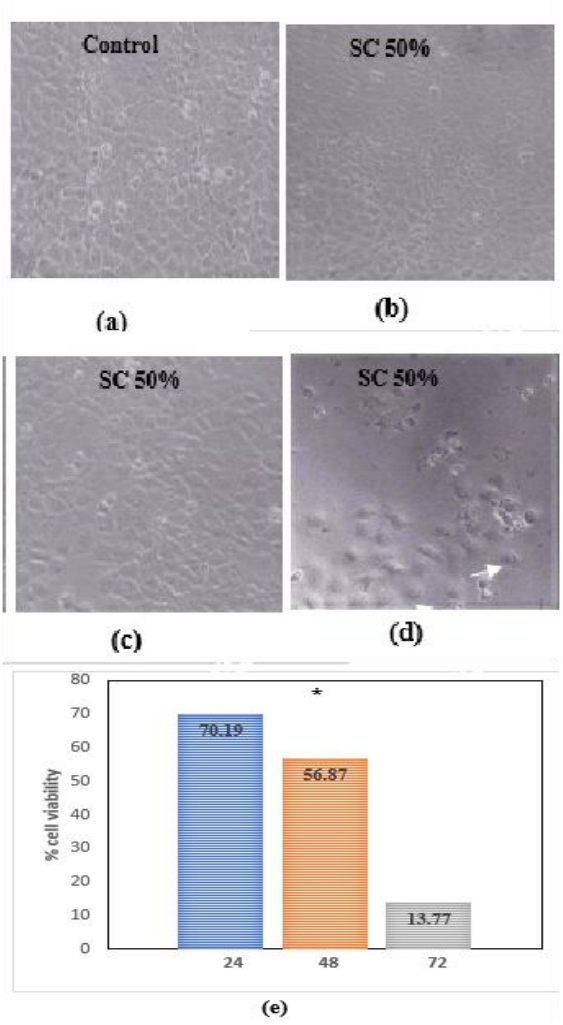

Figure 1: Morphology and cell viability of the cancer cells (mean ± SD; n = 3). * Symbols above bars represent statistical significance (*$p<0.05$). Cell morphology was observed under inverted microscop with 100 x magnification. Arrows show membrane blebbing and rounded cells.

Discussion

MSCs have emerged as a potential cell therapy for various of diseases including several types of cancer. Previus studies have been reported regarding the use MSCs in gastric cancer (20), ovarian cancer(21). However, using MSCs are still controversial whether it has the potential to suppress or promote cancer growth (22). Therefore, in the present study we use secretome as cell-free therapies over stem cell based therapis. Same with MSCs, secretomes have also been reported to either suppress or promote growth and development of cancer (23). Secretome has the advantages such as safer as they are devoid of viable cells, no risk of immunological reaction, can be produced, packaged and transported more easly and lower financial costs related with maintaining the cell (24). Furthermore, secretomes contain bioactive molecules to inhibit multiple hallmarks of cancer progression (25).

The interaction between secretome and cancer cell has not been fully understood. Be expected, secretome are able to increase p53 gene expression and decrease PI3K/AKT (26). Secretome modulate several pathways such as NF-κB, Wnt/β-catenin, Notch1, STAT3, and TGF-β which contribute to inhibit cell proliferation, migration, and angiogenesis (27-29).

We performed viability and proliferation assessments to verify the reduction of cancer cell characteristics after treatment by the secretome, and the results showed that the secretome derived from MSCs had an antiproliferative effect on MCF-7 breast cancer cells (Figure.1). This result are consistent with many reports suggesting that co-culture of UCMSCs and cancer cells in vitro had functions of proliferation inhibition of tumor cell(21). The direct impact of the secretome treatments in viability may be linked to cell death inducement or can be connected to the reduction in the proliferation capacity even though after 72h of treatment. This may be due to secretome inhibiting proliferation in a density dependent manner. Secretome will act by tumor cell contact inhibition(30). The morphological were observed at the meanwhile. The result showed treated cells were rounded up and cell to cell adhesion was lost (Figure.1d). These results are similar to the previous studies that reported restorative inhibition contact by secretome (31).

Conclusion

In conclusion, secretome has anticancer effect and could significantly inhibit cell viability and proliferation. These findings suggest that secretome is a promising candidate for breast cancer therapy. Further studies were needed to evaluate the mechanism of action secretome in inhibiting growth of breast cancer cell line.

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Conflict of interest

Non to declare

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