Effects of Chondroitin Sulfate (CS) on (HeLa) Cervical Cancer and Breast Cancer (MCF-7) Cell Lines

Mazma Syahidatul Ayuni Mazlan[1], Solachuddin Jauhari Arief Ichwan[2]*, Norhayati Rosli[1]

1. Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia.
2. Kulliyyah of Dentistry, International Islamic University Malaysia, Kuantan Campus, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia.

Abstract

Chondroitin Sulfate (CS) is the main constituent of Blue-spotted Stingray which shows promising in vitro anticancer activities in cancer cell lines. However, the effects of CS on human breast cancer and cervical cancer cell lines remain to be explored. Here we report that CS induced different degree of cytotoxicity in two human cancer cell lines, cervical cancer HeLa and breast cancer MCF-7 cell lines. We found that MCF-7 was more resistant to CS exposure than HeLa cell line. Moreover, CS induced apoptosis in HeLa but not MCF-7 cell line as shown by caspase-3 activity assay. The CS-induced caspase-3 activation in HeLa cells was also confirmed by using quantititative RT-PCR. Our findings show that the caspase-3 activation induced by CS in HeLa cells was transcriptional. These results indicate that as an anticancer candidate, CS is more potent on cervical cancer than the breast cancer cell line.

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Introduction

Cancer represents a major public health problem in the world.[1] In Malaysia, breast and cervical cancer are generally considered to be the most common malignancies among women.[2] According to Malaysian National Cancer Registry (MNCR) Reports 2007-2011, an estimated 56 713 new cancer cases diagnosed among women, with breast and cervical combined accounting for nearly one-third of new cases and also almost one-sixth of cancer death.[3]

Caspase-3 is a specific gene that plays a key role in the mitochondria pathway which initiated by caspase-9 and the death receptor pathway which involving caspase-8.[4] The terminal marker of the cell's entry point in both pathways is caspase-3.[5] Therefore, the activation of caspase-3 induces to apoptosis induction. However, previous researches show that MCF-7 cell line does not express caspase-3 due to deletion of *caspase-3* gene.[6]

Corresponding author:
Solachuddin Jauhari Arief Ichwan,
Kulliyyah of Dentistry, International Islamic University Malaysia, Kuantan Campus, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia.
E-mail: solachuddin@iium.edu.my

The importance of caspase-3 has been considered in the development of agents for cancer treatment. Generally, therapeutic measurements for treatment of cancer are surgery, radiotherapy and chemotherapy. However, these treatments have certain adverse side effects depends on the specific drugs and resistance to the treatment.[7] In addition, many of these options present limitation on anticancer activities.

Current investigations search for potent, safe and effective drugs to overcome resistance and reduce side effects. Many studies have been reported that the treatment of targeted cancer therapy has clearly arrived and proven to be highly successful in the treatment of cancer.[8] Thus, targeted therapy is recommended for cancer treatment since the treatment has fewer side effects compare to the traditional treatment procedures.

The blue spotted stingray is related to the main active components, Chondroitin Sulfate, or CS in short, a sulphated glycosaminoglycan (GAG) composed of repeating disaccharides of *N*-acetylgalactosamine and *D*-glucuronic acid with various sulfation patterns.[9] Many studies have been reported the role of CS as pharmacological drug via drug delivery system. On the other hand, CS has been shown to reduce the cancer growth without causing toxicity.[10]

However, despite knowledge of these potential anticancer drugs, the studies of the anticancer properties CS in breast and cervical cancer are still minimal. The purposed of the present investigation was to explore the mechanism of CS in inducing apoptosis on two different cell lines, HeLa and MCF-7.

Materials and methods

Cell lines

Two cancer cell lines, HeLa and MCF-7 were kindly provided by Prof. Dr. Masa-Aki Ikeda from Tokyo Medical and Dental University. The cells were cultured in DMEM (Gibco, Carlsbad, California, USA) medium and maintained in a humidified incubator with 5% CO_2 and 95% air at 37^{o}C.

Extraction and deproteinization

CS crude product was courtesy from Dr. Nina Suhaity Azmi from Universiti Malaysia Pahang. Blue-spotted stingrays were used for extraction of CS. The sample was reconstituted in ethanol for a week. After a week, the sample was weighed and blended with a mixture of urea and NaOH for deproteinization. Then, the sample centrifuged and the supernantant collected were lyophilized by freeze drying.

Cell viability assay

Cellular proliferation was assessed by the ability of cells to convert soluble MTT into an insoluble formazan precipitate. Exponentially growing cells were seeded in 96-well plates at an initial density of 2X104 / well, treated with defined concentrations of CS (10–100 µg/mL) After 24 hours incubation, the media were carefully removed and 30 µL MTT (Sigma-Aldrich, USA) at concentration of 5 mg/mL in phosphate buffer saline (PBS) was added to each well. After 2 hours incubation, the intracellular formazan complex was dissolved in DMSO and the absorbance was measured at 570 nm in a microplate reader. Cellular viability was expressed as a percentage of cell viability of CS-treated cells relative to untreated controls.

Caspase activity assay

Specific proteolytic activities of the caspase-3 in cells treated with CS were determined by the The Caspase-Glo® 3/7 Assay (Promega, USA). Cells were seeded in 96-well white plates and treated with either control (0.1% DMSO in DMEM). Then, the plates were analyzed using a luminometer at 6 hours intervals after exposure to CS up to 24 hours. Caspase-3 activities were measured and expressed as fold from the base controls.

RNA extraction, cDNA synthesis and quantitative RT-PCR

The RNA Extraction, cDNA Synthesis, oligonucleotide primers, and Quantitative RT-PCR setting conditions were performed as previously described with slight modifications11. Total RNAs were prepared using TRI Reagent (Sigma-Aldrich, USA). Complementary DNAs were synthesized from 5µg total RNA using the DiaStarTMKit (Solgent, South Korea) per the manufacturer's recommendations. Amplication was carried out in Rotogene-Q (Qiagen, Germany) with SYBR green detection and melt curve analysis. The amount of cDNA present in any given sample was normalized to the amount of DNA of the housekeeping gene β-actin. Reaction mixtures contained Master Mix, 300 nM each of forward and reverse final volume of 20 µL All samples were investigated in triplicates and then the melting curves obtained after PCR amplification confirmed the specificity of the SYBR green.

Statistical analysis

The data is obtained were expressed as the mean ± standard deviation (SD) that were interpreted using Microsoft Office Excel 2010® for Mac. The significance of differences was analysed by Student's t-test. A value of $p<0.05$ was considered significant.

Results

The present investigation examined the effects of CS on the viabilities of HeLa and MCF-7 cell lines by the MTT assay. The cells were treated with the defined concentration (10 – 100 µg/mL) of CS for 24 hours. The CS reduced the cell viability in a dose-dependent manner in both cell lines (Figure 1). We observed that CS was more cytotoxic to HeLa than MCF-7 cell line (IC50 50 µg/mL and 100 µg/mL in HeLa and

MCF-7, respectively). Next to further investigate whether the reduction of the cell viability by CS was due to apoptosis, we performed caspase-3 activity assay. While the caspase-3 activation was barely detected in MCF-7, we successfully demonstrated the activation of caspase-3 in HeLa cell line (Figure 2). Finally, to confirm whether the caspase-3 activation in the HeLa cells was transcriptional, we examined the transcription level of the caspase-3 mRNA. Indeed, as shown Figure 3, the caspase-3 mRNA in HeLa cells was upregulated following 6 hours of CS treatments as measured by real-time RT-PCR.

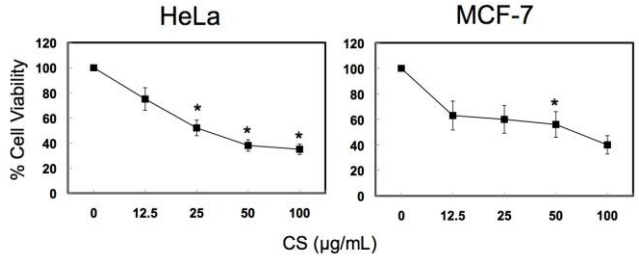

Figure 1. CS reduced the number of viable HeLa and MCF-7 cells in a concentration-dependent manner. Dose-response curve on the effect of CS analysed by MTT assay on HeLa and MCF-7 cell viability at 24 hours of exposure. The vertical and horizontal axes display percentage of cell viability and sample concentration (μg/mL), respectively. Data shown are means ± SD of three independent experiments each performed in triplicates. *p < 0.05 the comparison with the respective controls (0.1% DMSO).

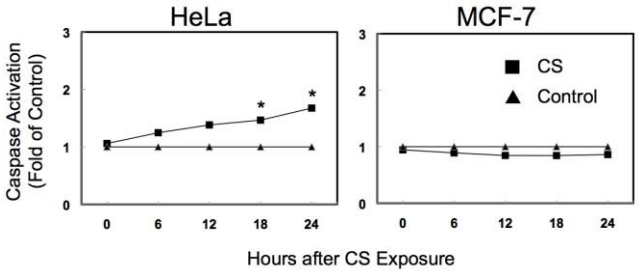

Figure. 2. Caspase-3 activity assays. The cells were treated with either 0.1% EtOH (control) or with CS for up to 24 hours and caspase-3 activities were quantified using luminescent assay. Each value is means ± SD of two separate experiments done in triplicate. *p < 0.05 by the comparison with the respective controls (0.1% DMSO).

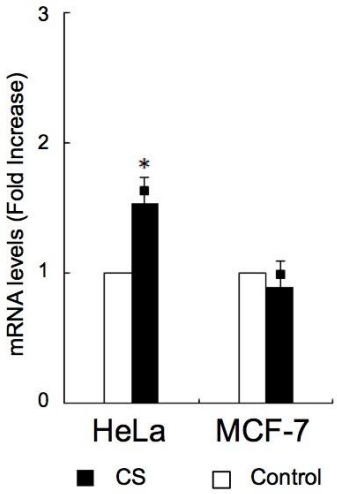

Figure 3. CS stimulates distinct apoptotic pathway in HeLa and MCF-7 cells determined by Quantitative RT-PCR. Expression levels of Caspase-3 mRNAs in the cells were quantified 6 hours following with either controls or with CS. Relative mRNA values depicted are means ± SD of two independent experiments each performed in triplicates. *p < 0.05 compared with respective controls.

Discussion

The current work used established cell culture models to study the anticancer mechanisms of CS extracted from the blue spotted stingray using HeLa and MCF-7 cell lines. Herein, the investigation revealed that CS significantly reduced the viability of both MCF-7 and HeLa cells in dose-dependent fashion. The cytotoxicity effect of CS was more evident in HeLa cells than MCF-7, suggesting that MCF-7 cel line was more resistant than HeLa to CS treatment. It is not surprising to notice this finding; MCF-7 cell line has been reported to exhibit resistance to a number anti-cancer candidates.[12,13]

To elucidate the mechanism by which CS reduced the viabilities of the cancer cells, the present study examined the effects of CS on apoptosis induction based on the activation of a widely used apoptotic marker, caspase-3. Caspase-3 has been established as an important factor among the components of the apoptotic machinery by orchestrating DNA fragmentation.[11] Recently, CS was found to induce apoptosis associated with activation of caspase-3 and its activation is fundamental to induce apoptotic cell

death.[10] The exposure of CS in HeLa cell line induced apoptosis as marked by elevated caspase-3 activation. In agreement to this, the caspase-3 mRNA expression also increased, suggesting that the CS-induced apoptosis in HeLa was mediated through caspase-3 pathway. On the contrary, both the level of caspase-3 activation and the mRNA transcription in MCF-7 cell line remained unchanged. This is reasonable because the caspase-3 gene is known to be deficient in MCF-7 cells[4,6] whereby the caspase-3 assay was the solely method used to detect apoptosis in this study.

Conclusions

We were unable to determine the nature of the viability reduction in MCF-7. However, we propose caspase-3 independent apoptosis or non-apoptotic cell death might involved. Further research is require to clarify the cell death pathway or mechanism.

Acknowledgements

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