The Effect of Brown Seaweed (*Sargassum sp.*) Extract on Apoptosis Process in Breast Cancer – A Literature Review

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Abstract

Breast cancer was one of the most common types of cancer in women and the major cause of death. Chemotherapy in breast cancer caused toxicity to the normal cells, resistance to the drugs, and several other side effects. Seaweed had many functional compounds that could be used as adjuvant therapy in breast cancer because of its anti-cancer properties called the proapoptotic agent. These compounds were fucoidan, phloroglucinol, and fucoxanthin. Their proapoptotic mechanisms shown by the journal were through increasing proapoptotic protein expression, decreasing antiapoptotic protein expression, inducing oxidative stress production on the cancer cell, and inhibiting the PI3K pathway. This review concludes that the brown seaweed (*Sargassum sp.*), which contains fucoidan, phloroglucinol, and fucoxanthin, could increase breast cancer cells' apoptotic process.

Keywords: *Apoptosis, Breast Cancer, Brown Seaweed,Oxidative Stress, Proapoptotic, Sargassumsp..*

Introduction

Breast cancer is the most prevalent cancer in women, there were 2.1 million women every year suffered breast cancer, and in 2018 around 627,000 women died from breast cancer(1). Nowadays, breast cancer's main treatments are surgery, chemotherapy, radiation, hormone therapy, and targeted therapy. These treatments are beneficial, although they can cause serious side effects such as nausea, bone marrow failure, resistance to drugs, and another significant clinical toxic effects(2,3). Recent cancer studies have focused on designing drugs from natural sources because of the fewer side effects than synthetic anti-cancer drugs. A new strategy has been proposed to reduce the dosage of synthetic drugs by providing a combination of natural compounds; one of them was seaweed. In China and Japan, several traditional medicines utilize seaweed to treat tumors(4,5).

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Sargassum is a brown seaweed genus with a tropical and subtropical distribution, which exists in all oceans. *Sargassum sp.* is a type of seaweed that is spread in Indonesia(6,7). Brown seaweed contains bioactive compounds such as fucoidan, phloroglucinol and fucoxanthin. Several studies show they have anti-cancer activity, through inducing apoptosis in cancer cells(4).

Apoptosis is a natural mechanism of cell death, and there are two mechanisms, the intrinsic pathway using mitochondrial proteins and the extrinsic pathway using extracellular signal induction. Apoptosis is very influential on cancer, so it is used as one of cancer treatment targets. Reactive Oxygen Species (ROS) or free radicals also play an essential role in the initiation, promotion and development of cancer, so antioxidants are needed to prevent cancer. But recent studies are interested in pro-oxidants as an anti-cancer therapy by inducing apoptosis with mitochondrial-dependent pathways and involvement of ROS production(8).This research was conducted to determine the effect of brown seaweed (*Sargassum sp*.) on the apoptotic process of breast cancer cells.

Methodology

Articles were collected using Pubmed and Science Direct database. The search words include apoptosis, breast cancer, brown seaweed, *Sargassum sp.*, oxidative stress, PI3K pathway, and proapoptotic. Articles were collected from the year 2015-2020 and indexed in Scimago and Scopus.

Effect of fucoidan, phloroglucinol, and fucoxanthin onapoptosis

Fucoidan is a fucose-enriched and sulfated polysaccharide obtained from the extracellular matrix of brown seaweed. Fucoidan has several anti-cancer mechanisms; one of them is inducing apoptosis[9]. Table 1 show some research about fucoidan anti-cancer activities. Fucoidan extract of *F. vesiculosus* was tested on different female cancer cell lines (breast, ovarian, uterine, endometrial carcinoma) in vitro. In most cancer cells, fucoidan treatmentdecreased in phosphorylated PI3K, AKT and mTOR. Fucoidan (*U. pinnatifida*) inhibits PI3K / AKT phosphorylation in prostate cancer cells in vitro. After treatment with fucoidan, the AML (Acute Myeloid Leukemia) cell line decreasedphosphorylated AKT by the in vitro method. The same decrease in AKT was seen in the other two AML cell lines (NB4 and HL60) when treated with fucoidan in vitro, derived from F. vesiculosus[10].

Phloroglucinol, one of the derivatives of phlorotannin has no toxicity but has a more protective role in normal tissue cells. It was found in a study that phloroglucinol showed anti-cancer properties throughupregulation of p53, Bax activation, Bcl-2 inhibition, increased caspase 3 and 9 activity and was associated with downregulation of the NF-kB line. Phloroglucinol can inhibit the growth of MCF-7 cells which induces apoptosisthrough the mitochondrial pathway with reactive oxygen species (ROS) production[4].

Fucoxanthin is major carotenoids produced by seaweeds, and their structure includes an allelic bond and an oxygenic functional group; these compounds have both protective and photosynthetic functions[11]. Several studies have reported that fucoxanthin's anti-cancer properties and its metabolites are pro-oxidants that can trigger apoptosis. A study has observed growth inhibition in the leukaemia cell lines by fucoxanthin and has linked it to ROS formation by fucoxanthin leading to apoptosis. There was an increase in H_2O_2 and O_2^- production due to treatment with fucoxanthin and the accumulation of cells containing sub-G_1 DNA content (indicating cessation of the cell cycle in stage G_1). In co-treatment with the commercial antioxidant NAC (N-acetylcysteine), the number of apoptotic bodies and cell DNA fragmentation decreased, which correlated the apoptotic effect fucoxanthin with the resulting ROS. Thus, they concluded that fucoxanthin's cytotoxic effect through ROSformation triggered apoptosis in HL-60 cells[12]. Several studies show fucoxanthin's anticancer activities in table 1.

Table 1. Effect of fucoidan, phloroglucinol, and fucoxanthin onapoptosis

Bioactive Compound	Method	Dosage	Sample	Result	Mechanism	Ref.
Fucoidan	In vitro	6.25, 12.5, and 25 µg/mL for 48 hours	MDA-MB-231 cell	Increase apoptosis process	Inhibit PI3K pathway	(13)
	In vivo	200 and 400 mg/kg.bw for 16 weeks	Sprague Dawley mice induced breast cancer			
	In vitro	0.8mg/mL for 48 hours	Melanoma B16 cell		Regulate apoptosis protein expression and increase ROS production	(14)
	In vivo	200 and 400 mg/kg.bw for 3 days	Sprague Dawley mice injected MCF-7 breast cancer cells		Modulate E-cadherin expression	(3)
	In vitro	50 and 150 µg/mL for 24 hours	MCF-7 cell		Activate caspase-8	(15)
	In vivo	500 and 1000 mg/kg.bw for 24 hours	HCC SMMC-7721 cell		Increase ROS production and activate caspase 3 and 9	(5)

Table 1. Effect of fucoidan, phloroglucinol, and fucoxanthin onapoptosis

Phloroglucinol	In vitro	10 and 30 µM for 48 hours	MCF-7 cell	Increase apoptosis process	Regulate apoptosis protein expression and activate caspase 3 and 9	(16)
	In vivo	25mg/kg.bw for 3 days	BALB / C mice injected breast cancer cells BT549 and MDA-MB231		Inhibit RAS signaling pathway	(4)
Fucoxanthin	In vitro	10 µM for 24 hours	MDA-MB-231 and MCF-7 cell	Increase apoptosis process	Regulate apoptosis protein expression and activate caspase pathway	(17)
	In vivo	200mg/kg.bw for 28 days	BALB / C mice injected Glioma U87 and U251 cell		Regulate apoptosis protein expression and inhibit PI3K/ AKT/m-TOR and p38	(18)
	In vitro	25 and 50 µM for 24 hours	Glioma U87 and U251 cell		Regulate apoptosis protein expression	

Role of oxidative stress on apoptosis

Oxidative stress refers to an imbalance between prooxidant and antioxidant factors controlled by many components; this imbalance can cause cell damage. ROS plays a vital role in oxidative stress and is produced as a byproduct of cellular metabolism, particularly in mitochondria. Once accumulated, ROS can attack cellular proteins, DNA, and lipids, leading to a state of oxidative stress. ROS contributes to several human diseases, including cardiovascular, inflammatory and neurodegenerative diseases, and cancer. Increased levels of mitochondrial ROS are sufficient to induce apoptosis[8].

The polysaccharide extract of *Sargassumwightii* was given to MCF-7 and MDA-MB-231 breast cancer cells. Polysaccharides significantly reduce the growth of both breast cancer cells, depending on the dose. The inhibition of polysaccharide growth against MCF7 cells was 69% at 500 µg / ml concentration. Furthermore, the inhibition rate of polysaccharide growth against MDA-MB-231 cells was 73% at 500 µg/ml concentration. The results showed polysaccharides induced apoptosis in breast cancer cells by increasing ROS formation, mitochondrial membrane division and nuclear destruction. Polysaccharides also increase caspase 3/9 activity, causing apoptosis in breast cancer cells. Based on this study, polysaccharides from *Sargassumwightii* can be candidates for further evaluation as anti-cancer agents for cancer in humans, particularly breast cancer[19].

Previous studies have reported the potential of polysaccharides from *Sargassumfusiforme* to induce apoptosis in leukaemia, stomach, bladder, and breast cancer cells. This study aims to determine *S. fusiforme* polysaccharides' effect on apoptosis and regulation of Human Erythroleukaemia (HEL) cells. The effect on HEL cell growth was detected by the Cell Counting Kit-8 (CCK-8) method, and apoptosis was detected by Hoechst staining. Cell cycle distribution and apoptosis were identified using flow cytometry. Cell cycle gene expression, p53, antiapoptosis gene, Bcl-xL and Bcl-2, and pro-apoptosis, Bax, Bad, and caspase-3 genes, as well as the corresponding protein expression, were detected using qPCR (quantitative Polymerase Chain Reaction) and western blot. The results showed that it decreased the viability of HEL cells and induced apoptosis of HEL cells. The apoptosis induction mechanism was found to be related to the formation of ROS, which in turn can lead to DNA damage and cell death, associated with induction of cell cycle arrest in the G_0 / G_1 phase, and increased expression of apoptosis-related genes and proteins[20].

Role of PI3K pathway on apoptosis

The PI3K signaling pathway is the center of the intracellular signaling axis that integrates multiple signals to induce cancer cells' growth and development. In general, the signaling path includes three main components consisting of PI3K, AKT and m-TOR. PI3K

is a kinase enzyme family that catalyzes PIP2 to form PIP3. PIP3 production promotes AKT phosphorylation as the primary regulator that can directly modulate several downstream targets, including the m-TOR and WNT / β-catenin pathways. AKT can regulate cancer cell proliferation, metabolism, apoptosis and angiogenesis. The m-TOR complex is activated by AKT and regulates several cell growth functions, including protein synthesis, cell survival, and autophagy inhibition. Recent data show that PI3K signaling is frequently activated in several malignancies in humans, including breast cancer and prolonged activation of PI3K signaling is associated with poor prognosis and resistance to chemotherapy[21].

Mutations in this signaling pathway are common in cancers, particularly in breast cancer. About 60% of tumours have genetic changes that cause hyperactivation of the PI3K / AKT / m-TOR pathway. Preclinical data from cell-based and genetic engineering studies (GEMs) have demonstrated that this mutation activates PI3K / AKT / m-TOR signaling and is an oncogenic driver by promoting cell transformation, tumour initiation, development, and resistance to apoptosis[22]. Cancer cells have developed strategies to avoid apoptosis, mutations in p53 and decreased expression of a molecule called caspase which is the crucial mediator and effector of apoptosis, disrupting external signaling and altering the balance between pro and anti-apoptotic molecules. Clinical studies in cancer have shown that targeting apoptosis is an effective strategy in fighting cancer. One strategy in targeting apoptosis is to target peripheral pathways effective in cell survival and apoptosis, such as PI3K-Akt-mTOR[23]. Several studies in table 1 support this strategy.

Conclusion

Brown seaweed (*Sargassum sp.*) which contains fucoidan, phloroglucinol and fucoxanthin can increase the apoptosis process in breast cancer with several mechanisms, through regulating apoptotic protein, increasing proapoptotic protein and decreasing anti-apoptotic protein, inducing oxidative stress (ROS) production and inhibiting the PI3K pathway.

Ethical Clearance – Not required since it is a literature review

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Conflict of Interest – Nil

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