

Gene Expression Study of Breast Cancer Cell in Response to Aloe Emodin Treatment

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Abstract. Breast cancer is the most common and cause of cancer death among Malaysian women. Despite many therapeutic advances in the treatment of this cancer, overall the incidence rate is still unacceptable. Mortality that results from the breast cancer is still alarming. There is emerging attention in the use of natural products as a new chemopreventive agent. Aloe emodin (Ae), a natural active compound present in Aloe Vera leaves has been reported to have several medicinal properties including antioxidant, antiviral, and anticancer activities. This paper provides a literature review of Ae with an insight on the experimental design to be used. This study is using microarray as a tool, and it is a hope that the transcriptional profiles might give a new understanding on how the Ae compound affect the gene alteration. Protein study also will be carried out to integrate with molecular findings that might provide basic rational for in vivo study or can be extended into clinical setting. The outcomes of this study may explore widened possibilities of the medicinal properties of Ae in other types of cancer.

Keywords: breast cancer, aloe emodin, anticancer.

1. Introduction

In recent years, breast cancer becomes the commonest cause of cancer related death in Asia [1]. National Cancer Registry of Malaysia provides an age standardized incidence rate (ASR) for 2004 of about 46.2 per 100,000 women. The rate however differs among three main races whereby the Chinese is the highest, followed by Indians and Malays [2]. Extensive studies in the last decade using microarray technology have provided better understanding of human cancer particularly in breast cancer. It has significantly showed a way to predict prognosis, treatment response and helping in making the clinical decision [3]. Therefore, we exploit the use of this tool to identify the effect of Ae in breast cancer cell lines. Proteomic study will also be carried out to further enhance our understanding from the underlying molecular mechanism to the functional stage of the cell. To date, no studies have been conducted to correlate between the effect of Ae in breast cancer cells with the variation in gene expression as well as the regulatory protein involved. Preliminary study [4] revealed that Ae has antiproliferative effect in breast cancer cell line.

2. Literature Review

2.1. Aloe Vera

Aloe is a genus and one of the widely known species is Aloe Vera or also known as *Aloe barbadensis* Miller. Ae is the well-known anthraquinone active compound that can be found in some species of Aloe [5]. Literature of different components found in Aloe species and their medicinal properties is shown in Table 1.

Table 1: Literature of different components and medicinal properties of Aloe Vera

Components of Aloe Vera	Medicinal properties	Author(s)
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High molecular fraction (barbaloin & polysaccharide)	• hypoglycemic effect on type 2 diabetes mellitus	[6]
Phytosterols	• hypoglycemic effect on type 2 diabetes mellitus	[7]
Polysaccharides	• immunity & antioxidant	[8]
Protein (14 kDa)	• antifungal & anti-inflammatory	[9]
Phenolic compounds	• protective & sealing effect against infection after wounding	[10]
Aloesin	• antioxidant, anti-inflammatory & anticancer	[11], [12]
Barbaloin	• anticancer	[12]
Glycoprotein	• wound healing	[13]

Numerous studies also reported on medicinal properties derived from different extracts of Aloe Vera. The pharmacological elements of this species are studied extensively via in vivo and in vitro as well. Summary of the literature is shown in Table 2.

Table 2: Literature on medicinal properties extract of Aloe Vera

Type of extract	Medicinal properties	Type of study	Author(s)
Pulp	detoxification agent	in vivo	[14]
Pulp & liquid fraction	antifungal	in vitro	[15]
Pulp	anticancer	in vivo	[16]
Gel	wound healing	in vivo	[17], [18]
Gel	antimicrobial	in vitro	[19]
Gel	anti-inflammatory	in vitro, in vivo	[20],[21]
Aerial parts	hepatoprotective	in vivo	[22]

2.2. Aloe Emodin

Various studies conducted have revealed that Ae can induce cell death to various cancer cell lines. Table 3 provides the literature on the anticancer properties of Ae from different type of human cancer cell lines.

Table 3: Literature on various mechanism of anticancer properties of AE

Type of cancer	Type human cell line	Various mechanism of anticancer properties of AE	
Cervical	KB	• induces in vitro G ₂ /M arrest and activates alkaline phosphatase.	[23]
	HeLa	• involves G ₂ /M arrest and induce differentiation	[24]
Lung	H460	• photosensitized AE-induced anoikis associated with protein expression of α -actinin & mitogen-activated protein (MAP) kinase members.	[25]
	H460	• induces apoptosis through involvement of modulation of cAMP-dependent protein kinase, protein kinase C, Bcl-2, caspase-3 and p38 protein expression.	[26]
Gastric/ Stomach	CH27 & H460	• induces apoptosis through involvement of protein kinase C	[27]
	AGS & NCI-N87	• releases apoptosis-inducing factor & cytochrome C from mitochondria, activates caspase-3 that lead to nuclear shrinkage & apoptosis.	[28]
	MGC-803 & SGC-7901	• involves in suppression of c-myc expression	[29]
	MGC-803	• induces growth inhibitory effect with an increase in S phase and alkaline phosphatase activity repression	[30]
Nasopharynx	NPC-TW 039 & NPC-TW 076	• induces G ₂ /M phase arrest by increase cyclin B1 level bound to Cdc2	[31]
Liver	Hep G2 & Hep 3B	• has antiproliferative activity through p53-dependent and p21-dependent apoptotic pathway	[32]
	Hep G2	• affects multiple proteins associated with oxidative stress, cell cycle arrest, antimetastasis, and hepatitis C virus replication	[33]
Brain	SVG & U-373MG	• modulates PKC isozymes, inhibits proliferation & induces apoptosis	[34]
Blood- leukemia	HL-60	• induces in vitro G ₂ /M arrest of cell cycle	[35]
Bladder	T24	• induces apoptosis through the p53 dependent apoptotic pathway	[36]
Tongue	SCC-4	• induces cell death through S-phase arrest and apoptosis	[37]

3. Research Framework

It was previously found that Ae has significantly showed antiproliferative activity in human breast cancer cell line, MCF-7. Therefore, this study aimed to screen the genes and proteins that may play a role in Ae-suppressed proliferation of breast cancer cells. Further understanding and identifying the role of these genes and proteins will enable us to understand the regulation of its metabolic activity. This study hypothesized that Ae regulates the genes and proteins in the suppression of breast cancer cells proliferation. The following figure 1 represents the research framework and the explanation of each of the phases.

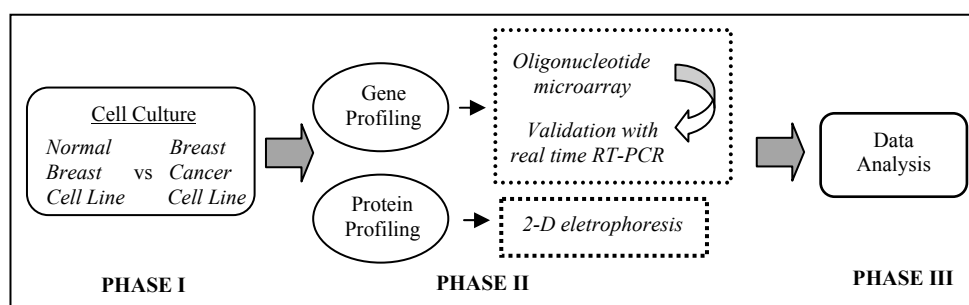


Fig. 1: Research Framework.

3.1. Phase I

Breast cancer cell line (MCF-7) and normal breast cell line (MCF-10A) will be cultured in complete DMEM and DMEM/HAM's F-12 medias (FLOWLAB, Sydney, Australia), respectively as monolayer to approximately 80% of confluence in 5% CO₂, at 37°C. All cells will be purchased from American Type Cell Collection (USA). Cells will be treated with aloe emodin at IC₅₀ value for 48 hours. Total RNA from cells (MCF-7-and MCF-10A) will be isolated with the RNeasy Mini Kit (Qiagen, Stanford, CA). All samples will be processed following the manufacturer's directions. For protein extraction, the same group of samples will be harvested and disrupted following the standard protocol of protein isolation.

3.2. Phase II

The extracted mRNA will be converted to biotin-labeled complementary RNA using The MessageAmp aRNA kit (Ambion Inc, USA). It then will be hybridized to HG-U133A Affymetrix oligonucleotide microarrays (Santa Clara, CA). The arrays will be washed and scanned according to standard Affymetrix protocol (www.affymetrix.com). From the microarray results, about 20 genes will be chosen for further validation. A real time RT-PCR will be carried out to justify the microarray analysis. For protein study, the first-dimensional isoelectric focusing (IEF) will be performed followed by second-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, in-gel proteolytic digestion will be carried out following mass analysis using the MALDI-ToF MS. Spots of the MALDI ProteinChip array with hydrophobic surface will be pretreated with 2uL of 50% acetonitrile containing 0.5% of trifluoroacetic acid (TFA). Protein will be identified by searching in publicly available NCBI database (www.proteomicmetrics.com) using the peptide mass fingerprint generated by trypsin digestion.

3.3. Phase III

Microarray data will be analyzed using GeneSpring GX software. Protein identification will be determined using mass spectrometry and database searching.

4. Conclusion

This paper provides extensive literature on Ae as anticancer agent besides giving an insight of the research framework to be carried out. There are two ways in assessing the gene expression profile either by measuring the final product which is protein or its intermediate, the mRNA. DNA or oligonucleotides microarrays have been used widely for the analysis of transcription study; however expression in mRNA is not necessarily reflected to the changes at protein level. Monitoring on the mRNA level is not sufficient to provide significant information on the various modifications occurred during post-translational stage, which is crucial for protein functions. Therefore, this study was designed to tackle the challenge whereby gene and protein profiling will be carried out concurrently. The findings of the expected results from this experiment may provide indications of the differential expression of gene and protein that may reveal underlying mechanism of Ae in breast cancer cell lines thus suggesting the Ae as a promising candidate for natural chemoprevention agent in breast cancer treatment.

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