THE EFFECTS OF 2-METHOXY-1,4-NAPHTHOQUINONE (MNQ) ON GLUCOSE METABOLISM OF MDA-MB-231 CELLS

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by

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LIST OF ABBREVIATION

cm^2	centimeter squared
h	hour
kDa	kilodalton
L	litre
MV	megavoltage
mA	miliamphere
mg	miligram
min	minute
ml	mililitre
ng	nanogram
sec	second
^oC	degree celcius
µg	microgram
µl	microlitre
µM	micromolar
V	voltage
ATCC	American Type Cell Culture
ATP	adenosine triphosphate
CO_2	carbon dioxide
Ct	threshold cycle
Ca^{2+}	ion calcium
cDNA	complementary deoxyribonucleic acid

DCIS	ductal carcinoma in situ
DNA	deoxyribonucleic acid
ER	estrogen receptor
FITC	fluorescein isothiocyanate
GLUT	glucose transporters
HIF	hypoxia-inducible factors
HKII	Hexokinases II
H_2O_2	hydrogen peroxide
IHC	immunohistochemical
IC_{50}	half maximal inhibitory concentration
LCIS	lobular carcinoma in situ
LC3	microtubule-associated protein light chain 3
LDH	lactate dehydrogenase
MNQ	2-Methoxy-1,4-Naphthoquinone
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mRNA	messenger ribonucleic acid
mTOR	mammalian target of rapamycin
NAD^+	nicotinamide adenine dinucleotide
PR	progesterone receptor
RT-PCR	real time polymerase chain reaction
ROS	reactive oxygen species
rpm	revolutions per minute
TAM	tamoxifen
TNBC	triple negative breast cancer

KESAN 2-METOKSI-1,4-NAFTOKUINON (MNQ) DALAM METABOLISME GLUKOSA SEL-SEL MDA-MB-231

ABSTRAK

Kuinon dan bahan terbitannya telah menunjukkan aktiviti biologi dan sifat perubatannya termasuk antikulat, antibakteria dan antikanser. Sehingga kini, para penyelidik telah memfokuskan sasaran ubat kemoterapi terhadap gen atau protein/enzim yang spesifik dalam metabolisme kanser. Kajian ini menggunakan 2-metoksi-1,4-naftokuinon (MNQ) berdasarkan peningkatan laporan kesan perubatannya dalam mendorong kematian sel kanser. Kajian ini bertujuan untuk menilai kesan MNQ dalam metabolisme glukosa kanser payudara tigaan negatif, sel-sel MDA-MB-231. Pada permulaannya, sel-sel MDA-MB-231 dirawat dengan pelbagai dos MNQ untuk menentukan kesan perencatan pertumbuhan dan dos IC_{50}. Kesan MNQ juga telah diuji di dalam sel-sel epitelium payudara bukan malignan, MCF10A. Untuk analisis lanjutan, sel-sel MDA-MB-231 telah dirawat dengan MNQ pada dos IC_{50} untuk pengukuran pengambilan glukosa, penghasilan laktat serta pengekspresan molekul yang terlibat dalam glikolisis (GLUT1, Akt, HKII dan HIF1α). Kesan MNQ juga telah diuji pada aktiviti autofagi dan pengekpresan gen yang terlibat dengan autofagi (Beclin 1 dan LC3). Hasil kajian menunjukkan bahawa MNQ menyekat pertumbuhan sel-sel MDA-MB-231 dengan nilai IC_{50} 43, 29 dan 22 μM (24, 48 dan 72 j rawatan) tetapi tidak pada sel-sel MCF10A. MNQ juga menyekat aktiviti glikolitik pada sel-sel MDA-MB-231 melalui perencatan pengambilan glukosa dan pengurangan penghasilan laktat. Penurunan pengawalaturan GLUT1 dan Akt pada kedua-dua gen dan protein di dalam

sel-sel MDA-MB-231 menunjukkan bahawa perencatan glikolisis oleh MNQ adalah melalui perantaraan isyarat laluan GLUT1/Akt. Peningkatan pengekspresan gen HKII, Beclin 1, LC3 dan pembentukan autofagosom di dalam sel-sel MDA-MB-231 menunjukkan MNQ mengaruh aktiviti autofagi. Perencatan pengambilan glukosa ke dalam sel-sel mungkin menyebabkan kekurangan nutrien lalu mengaktifkan autofagi di dalam sel-sel MDA-MB-231. Sebagai kesimpulan, kebolehan MNQ untuk menghalang glikolisis melalui sasaran ke atas molekul yang terlibat dengan glikolisis di dalam sel-sel kanser payudara tigaan negatif (MDA-MB-231), mencadangkan potensi MNQ sebagai agen antikanser /adjuvan untuk rawatan kanser payudara.

THE EFFECTS OF 2-METHOXY-1,4-NAPHTHOQUINONE (MNQ) ON GLUCOSE METABOLISM OF MDA-MB-231 CELLS

ABSTRACT

Quinone and its derivatives are shown to have biological activities and medicinal properties including antibacterial, antifungal as well as anticancer activities. To date, the researchers have been focusing on targeting the chemotherapeutic drugs at the specific genes or proteins/enzymes of cancer metabolism. In this study, 2-Methoxy-1,4-Naphthoquinone (MNQ) was selected based on the increasing reports of its medicinal effects in promoting cancer cells death. The aim of this study is to evaluate the effects of MNQ on glucose metabolism in triple-negative breast cancer, MDA-MB-231 cells. Initially, the MDA-MB-231 cells were treated with various doses of MNQ to determine the growth inhibitory effects and the IC_{50} dose. The effect of MNQ on cell growth was also tested in non-malignant breast epithelial cells, MCF10A. For further analyses, the cells were treated with IC_{50} dose of MNQ for measurement of glucose uptake, lactate production as well as the expression of glycolysis-related molecules (GLUT1, Akt, HKII and HIF1α). The effect of MNQ was also tested on the autophagy activities and the expression of autophagy-related genes (Beclin 1 and LC3). The results showed that MNQ inhibited the proliferation of MDA-MB-231cells with IC_{50} values of 43, 29 and 22 μM (24, 48 and 72 h of treatment) but not in MCF10A cells. MNQ also inhibited the glycolytic activities in MDA-MB-231 cells by inhibiting the glucose uptake and reducing the lactate production. The down-regulation of GLUT1 and Akt in both gene and protein expression of MDA-MB-231 cells suggested that inhibition of gycolysis by

MNQ was mediated by GLUT1/Akt signaling pathways. Increased HKII, Beclin 1 and LC3 gene expression as well as the formation of autophagosomes in MDA-MB-231 cells showed that MNQ induced the autophagy activity. The inhibition of glucose uptake into the cells may cause the nutrient deprivation and lead to the activation of autophagy in MDA-MB-231 cells. In conclusion, the ability of MNQ to inhibit glycolysis by targeting the glycolysis-related molecules in triple-negative breast cancer cells (MDA-MB-231), indicated the potential of MNQ as anticancer agent/adjuvant in breast cancer treatment.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEWS

1.1 Cancer

Cancer is a class of disease characterized by uncontrolled cell proliferation and has the ability to spread throughout the whole body. Cancer that grows locally known as benign tumor, whereas the other one, malignant tumor can invade and spread into nearby tissues and the process is known as metastasis (Sudhakar, 2009, Baskar et al., 2014). Cancer cells are formed due to the modification or mutation of the DNA of the normal cells, either by spontaneous or induced by environmental factors such as radiation, chemicals and microorganisms. Cancer cells continue to develop if the body immune system is not functioning properly and worse under certain condition such as unhealthy environment, poor diet and people at an advanced age (Sharma et al., 2010).

1.1.1 Overview of cancer incidence

The most common cancers in both men and women are lung, followed by breast, colorectal and prostate cancer. Cancer is the second leading cause of death worldwide, and the numbers are expected to increase more than two times in the next 20-40 years, surpass the current leading cause of death, heart disease (Bray et al., 2018, Jemal et al., 2010, Thun et al., 2009, IARC, 2018). In 2018, an estimated 18.1 million new cancer cases and 9.6 million cancer deaths were recorded worldwide, which lung cancer, leading the number with a total of 11.6 % of cancer diagnosed and 18.4 % cancer death

(Bray et al., 2018, IARC, 2018) (Figure 1.1). International Agency for Research on Cancer (IARC) has projected that by the year 2030, about 26 million new cases and 17 million of death caused by cancer will occur each year all over the world (IARC, 2018).

1.1.2 Cancer incidence in Malaysia

Asia is a diverse continent and accounts for 60 % of global population (Bray et al., 2018). Most Asian countries are still developing except for high-income countries (HICs) such as Japan, Hong Kong, South Korea, Singapore, United Arab Emirates, Kuwait, Qatar, Saudi Arabia and Bahrain. The majority of the population in Asia is grouped as low and middle-income countries (LMICs) for example India, Philippines, Thailand and including Malaysia. Consequence of the diverse economic development and investment in HICs and LMICs, Asian countries have highly variable health service development and healthcare infrastructure (Sankaranarayanan et al., 2014). Compared to HICs, most LMICs have poor, limited health services and the cancer burdens are significant in these areas. Other than that, increasing urbanization, changing lifestyle, obesity, diet and increasing lifespan also contribute to the increasing cancer burden and changing cancer pattern in different regions of Asia (Forman et al., 2014, Sankaranarayanan et al., 2014, Długosz and Raźniak, 2014).

Asia accounts for almost half of the cancer burden which is 48.4 % of cancer cases with estimated 8.7 million, whereas 5.4 million deaths (57.3 %) in 2018. More than 70 % of the cancer deaths were reported to occur in LMICs. These incidences of cancer cases and cancer deaths are estimated to increase continuously to over 10.7 million and 7.5 million respectively in 2030 (Ferlay et al., 2010, IARC, 2018).

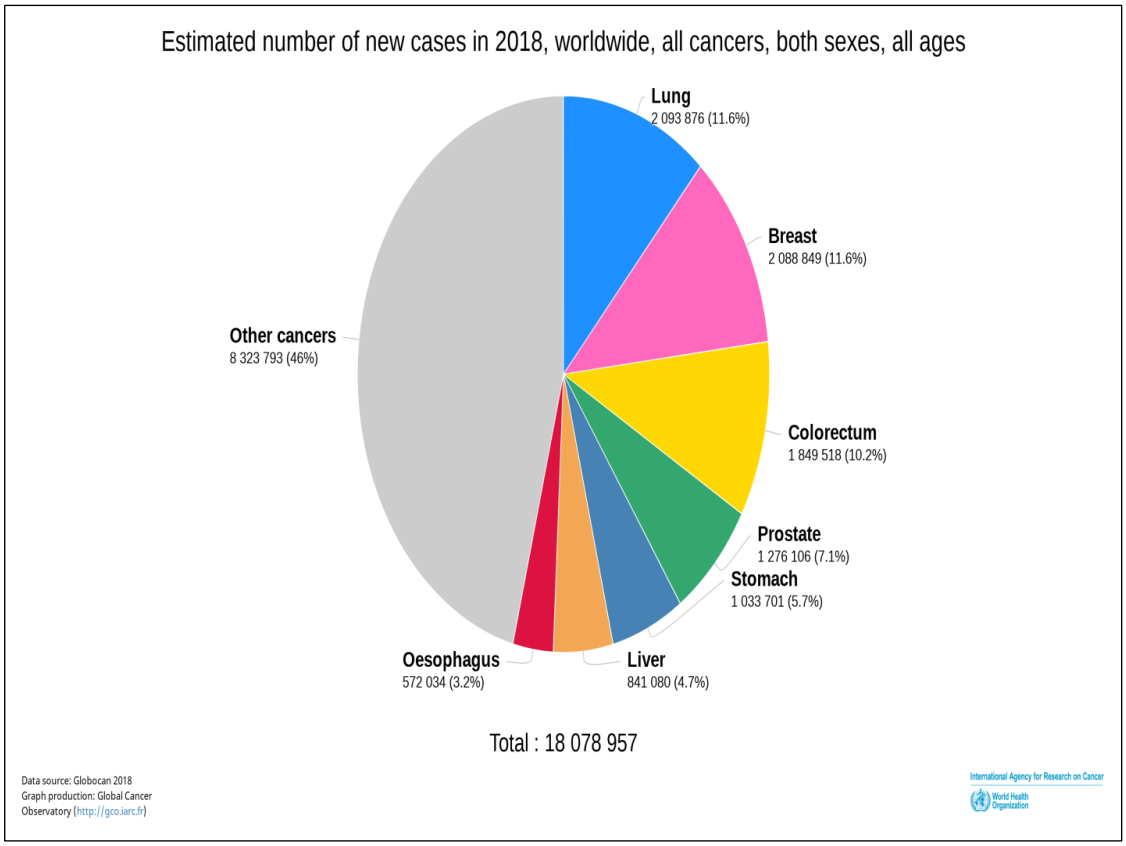

Figure 1.1 Estimated number of new cases in 2018 both in men and women worldwide

Over 18 million new cancer cases were estimated in 2018 both in men and women worldwide with lung cancer recorded the highest cases followed by breast, colorectum, prostate and stomach. (Extracted from http://gco.iarc.fr/today/home).

In Malaysia, 103,507 new cancer cases and 64,275 cancer deaths were reported in the year of 2007-2011, which increased by fivefold from data recorded in 2003 (21,464 cancer cases) according to the National Cancer Institute (NCI, 2016). In 2018, breast cancer is the most common cancers diagnosed in Malaysia which accounts for 17.3 % of new cancer cases (7,953) and 11.0 % of cancer death (2,894) both in men and women (IARC, 2018).

1.2 Breast cancer

Cancer is named according to the body part in which the cancer originated. For example, sarcomas are cancer from muscle and connective tissue, whereas carcinomas are cancers derived from epithelial cells (Alberts et al., 2007, Heidari, 2019). Thus, breast cancer refers to the uncontrolled growth of the cells that originate from breast tissue, most commonly from the inner lining of the milk ducts or the lobules that supply the ducts with milk (Sharma et al., 2010). Breast cancer is the most frequently diagnosed life-threatening cancers and most common cause of cancer death in women worldwide. In 2018, almost 2.1 billion new cases of breast cancer were reported in both men and women, and nearly 627,090 of death occurred due to breast cancer worldwide (IARC, 2018).

1.2.1 Risk factors for developing breast cancer

There are many factors that can contribute to cancer development. Generally, cancers are caused by dietary habits, lifestyle and environmental factors (Kamińska et al., 2015, Sun et al., 2017). Breast cancer is believed to be caused by a single or a combination of intrinsic (age, sex, familial susceptibility and hormonal changes) and extrinsic (dietary

habits, physical activity) factors. Among all, age and female sex have been considered as a major risk for developing breast cancer where it was frequently observed in women at menopausal age. The incidence of breast cancers was recorded higher among women between 40 and 59 years and it reaches a plateau in women at age more than 70 (Kamińska et al., 2015). Besides, breast cancer also is a type of cancer that is closely associated to the hormonal status in the body. Thus, the chances of women getting breast cancer are higher compared to men due to the cumulative lifetime exposure to estrogen, such as puberty, pre-menopausal monthly cycles, pregnancy and breastfeeding (Davis and Lin, 2011, Key et al., 2013). It was also reported that breast cancer incidence is due to genetic heredity factors and related to family history. Women who had a first degree relative with breast cancer have higher risk of developing breast cancer compared to women without any affected relative (Brewer et al., 2017). The inherited susceptibility to the breast cancer is partially attributed to the mutations of breast cancer related genes such as BRCA1 and BRCA2 (Sun et al., 2017).

1.2.2 Types of breast cancer

The compositions of the breast are mainly from the two types of tissues which are glandular tissues and stromal tissues. Glandular tissues contain milk-producing glands or known as lobules and ducts. Meanwhile, stromal tissues is composed of fatty and fibrous connective tissues to support the breast (Sharma et al., 2010) (Figure 1.2). Breast cancer has been grouped into different subgroups based on the histological appearance, stage, tumor grade, lymph node status and molecular profiles. The heterogeneity of breast cancer makes diagnosis and treatment become more challenging (Holliday and Speirs, 2011, Polyak, 2011).

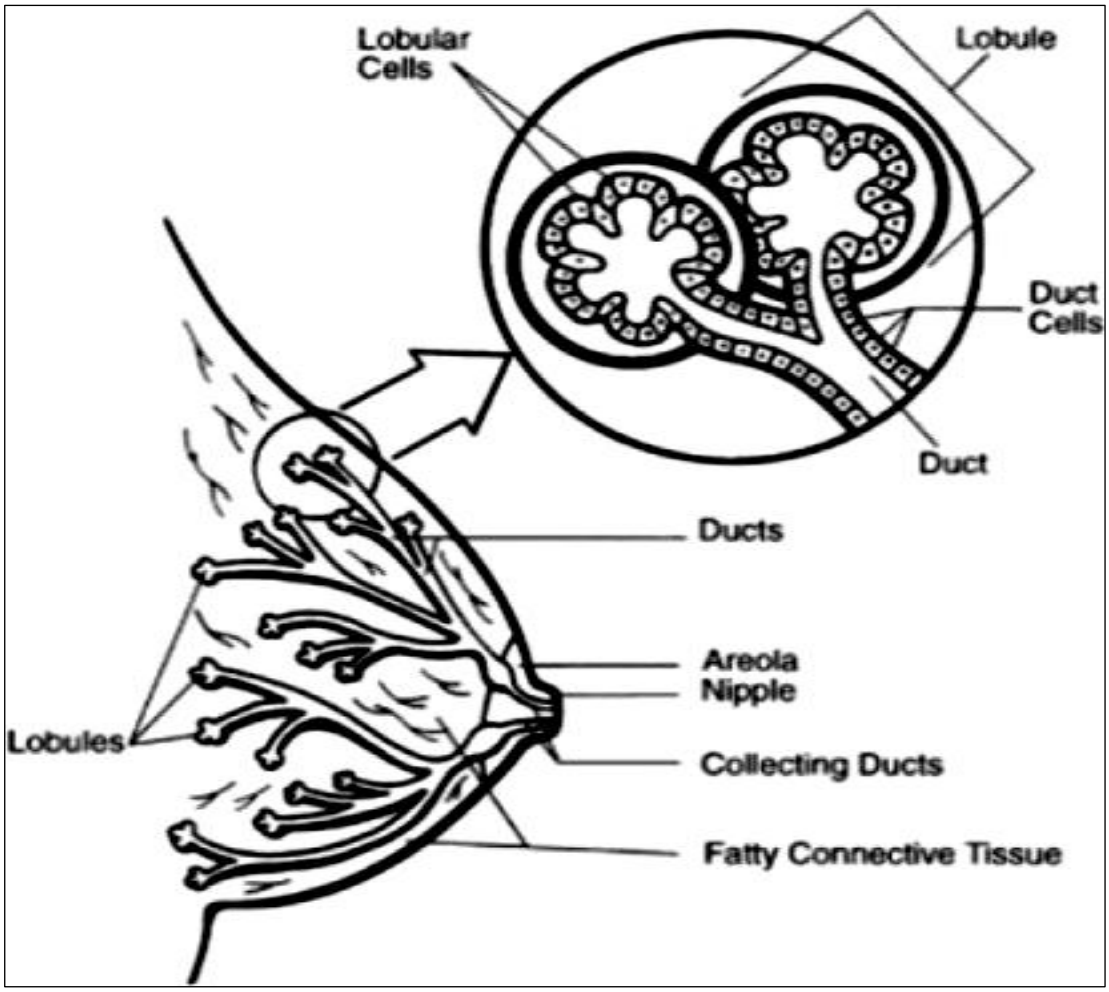

Figure 1.2 The structure of breast

Breast cancer usually develops from the inner lining of milk duct or the lobules that supply the ducts with milk. Ductal carcinoma refers to breast cancer originating from ducts and those originating from lobules are known as lobular carcinoma. (Extracted from Sharma et al., 2010).

In general, breast cancer can be broadly classified into 2 types; carcinoma *in situ* and invasive carcinoma (Table 1.1).

All carcinomas are grouped as ductal and lobular based on the site from which the tumor arises. For example, cancers originating from the ducts are referred as ductal carcinomas, whereas those originating from the lobules are referred as lobular carcinomas (Makki, 2015, Nounou et al., 2015).

Carcinoma *in situ* is classified as either ductal (ductal carcinoma *in situ*) DCIS or lobular (lobular cancer *in situ*) LCIS. DCIS often found in the mammary duct, while LCIS arise from atypical lobular hyperplasia. Both *in situ* carcinomas arise from atypical proliferation of cells and do not invade normal tissue, thus they cannot cause serious morbidity unless they become invasive (Logan et al., 2015).

Invasive carcinoma refers to malignant abnormal proliferation of neoplastic cell in the breast tissue, which has penetrated through the duct wall into stroma. The major invasive tumor types include invasive ductal carcinoma (IDC) or invasive lobular carcinoma (ILC). The IDC is the most common type of breast cancer and accounts for 70-80 % of breast cancer incidence reported (Lester et al., 2009). Most of the breast malignancies are adenocarcinoma, which constitutes more than 95 % of breast cancers (K et al., 2010).

1.2.3 Molecular classification of breast cancer

Study by Perou et al. (2000) has classified breast cancer into the subgroups according to the similarities in the gene expression profiles using the microarray technique. This classification technique was widely accepted by the medical and scientific community

Table 1.1 Types of breast cancer (Adapted from Makki, 2015 and Nounou et al., 2015).

Types of breast cancer	Description
Ductal carcinoma *in situ* (DCIS)	Begin inside the milk ducts of the breast and do not spread to other area.
Lobular carcinoma *in situ* (LCIS)	Drastic increase in the number of cells within the lobules of the breast and do not spread to other area.
Invasive ductal carcinoma (IDC)	Begin in the milk ducts of the breast and penetrates the wall of the duct, invading the fatty tissues of the breast and possibly other regions of the body. The most common types of breast cancer, accounting for 80 % of breast cancer diagnosis.
Invasive lobular carcinoma (ILC)	Begin in the milk glands (lobules) of the breast, but spread to other regions of the body. Account for 10-15 % of breast cancer.

with the hope that this technique will provide a new insight into the biology of breast cancer, thus determine a variety of therapeutics approach in targeting breast cancer. The classification of breast cancers is categorized based on the gene signature of estrogen receptor (ER), progesterone-receptor (PR) and human epidermal growth factor receptor 2 (HER2) (Tang and Tse, 2016) as summarised in Table 1.2.

Luminal A

Luminal A is characterised by ER/PR positive and HER2 negative. It includes a wide range of low-grade variants and account for 50 % of invasive breast cancer. Luminal A basically has a good prognosis for treatment (Inic et al., 2014).

Luminal B

Luminal B is the ER/PR positive and HER2 is variable (positive or negative). The proliferation rate and histological grades are higher than luminal A, which moderate expression of hormone receptor and associated genes. This category account 20 % of invasive breast cancer diagnosed and the response to the therapy is variable. Luminal B prognosis is lower compared than luminal A (Inic et al., 2014).

HER2 overexpression

HER2 overexpression is ER/PR negative, but HER2 strong positive. This tumor is more likely to be of high grade, particularly aggressive and more likely to spread rapidly than other types of breast cancer. This group of breast cancer compromises 15 % of all invasive breast cancer and implies poor prognosis. It was shown to have the highest sensitivity against trastuzumab (herceptin) therapy (Logan et al., 2015).

Table 1.2 Classification of breast cancer based on molecular expression (Adapted from Holliday and Speirs, 2011).

Classification	**ER**	**PR**	**HER2**	**Other characteristics**
Luminal A	Positive	Positive/ Negative	Negative	Ki67 low, endocrine responsive, often chemotherapy responsive
Luminal B	Positive	Positive/ Negative	Positive	Ki67 high, usually endocrine responsive, variable to chemotherapy. $HER2^+$ are trastuzumab responsive
HER2 overexpression	Negative	Negative	Positive	Ki67 high, trastuzumab responsive, chemotherapy responsive
Basal-like (Triple negative)	Negative	Negative	Negative	$EGFR^+$ and/or cytokeratin $5/6^+$, Ki67 high, endocrine nonresponsive, often chemotherapy responsive
Claudin low	Negative	Negative	Negative	Ki67, E-cadherin, claudin-3, claudinin-4 and claudinin-7 low. Intermediate response to chemotherapy

Basal-like breast cancer

The basal-like breast cancers are named according to its pattern that is similar to the basal epithelial cells. It is ER/PR negative and HER2 negative (triple negative) and referred to triple-negative breast cancer (TNBC). TNBC cell lines are highly aggressive, metastatic and typically lack expression of the molecular targets that confer responsiveness to highly effective targeted therapies such as tamoxifen or trastuzumab. It has generally poor prognosis and compromises about 15 % of all invasive breast cancers (Toft and Cryns, 2011).

Claudin low

Claudin low is a more recently added class with triple negative (ER, PR and HER2). However, only a minority of TNBC are Claudin low. This subtype was distinct to basal like by the low expression of cell-cell junction protein, including claudins 3, 4, 7, and E-cadherin (Herschkowitz et al., 2007, Nounou et al., 2015).

1.2.4 Stages of breast cancer

Since 2018, the stages of breast cancers are determined according to the updated staging guidelines by American Joint Committee on Cancer (AJCC). Considering the additional information on the tumor grade, ER, PR, and HER2 status have made determining the stage of breast cancer more complicated, but also more accurate. The stages of breast cancer are expressed in the Roman numerals, starting from stage 0-IV. Stage 0 is considered as non-invasive or in situ cancer stage, whereas I is the (invasive) least advanced stage and continue with stage II, III and IV with more advanced stages as listed in Table 1.3.

Table 1.3 Stages of breast cancer (Adapted from https://www.breastcancer.org/symptoms/diagnosis/staging#stage0).

Stage		**Tumor grade**	**ER**	**PR**	**HER2**
0		DCIS; cancer cells do not invade neighboring tissues	None	None	None
I	IA	The tumor size > 2 cm Not spread outside the breast No lymph nodes involved	Positive	Positive	Negative
	IB	No tumor in breast Tumor between 0.2 mm to 2 mm- found in lymph nodes, or Tumor in breast < 2 cm Tumor between 0.2 mm to 2 mm-found in lymph nodes	Negative	Negative	Negative
II	IIA	No tumor in breast Tumor > 2 mm found in 1-3 axillary lymph nodes or lymph nodes near the breast bone, or Tumor < 2 cm has spread to the axillary lymph nodes, or Tumor between 2 cm-5 cm and has not spread to the axillary lymph nodes	Negative	Positive	Positive
	IIB	Tumor in breast between 2 cm-5 cm Tumor between 0.2 mm to 2 mm-found in the lymph nodes, or Tumor between 2 cm-5 cm has spread to 1-3 axillary lymph nodes, or Tumor > 5 cm but has not spread to axillary lymph nodes	Negative	Negative	Negative

Table 1.3 continued

III	IIIA	No tumor in breast Tumor is found in 4-9 axillary lymph nodes, or Tumor > 5 cm Tumor between 0.2 mm-2 mm-found in lymph nodes, or Tumor > 5 cm Tumor has spread to 1-3 axillary lymph nodes	Negative	Negative	Negative
	IIIB	Tumor may be any size has spread to the chest and caused swelling or ulcer, and May spread to 9 axillary lymph nodes	Negative	Negative	Negative
	IIIC	Reddening around breast area Breast feels warm and swollen Cancers have spread to the lymph nodes	Negative	Negative	Positive
IV		Metastatic breast cancer; invasive breast cancer that has spread beyond breast area and nearby lymph nodes to the other organs of the body	None	None	None

1.2.5 Breast cancer management

In recent years, many treatment and management options exist for cancer patients and resulted in wide variations in the survival and mortality among breast cancer patients in different countries and population worldwide. Many factors influenced these variations such as breast cancer awareness, early detection, intervention, better medical therapies and improved surgical techniques. Despite these factors, breast cancer remains the most common cancer cause of death from cancer in women worldwide (Hortobagyi et al., 2005, Kingston and Johnston, 2016).

Screening mammography has been recorded as the greatest contribution to early detection and decrease in breast cancer mortality. Mammography is the preferred screening examination for detecting abnormality of the breast before it can be felt by the woman herself or her physician. The patient feels a breast lump when breast cancer has grown to the point where physical symptoms and signs appear. Mammography is widely available, well-tolerated and the price for the test are affordable (Løberg et al., 2015). Once breast cancer is diagnosed and localized, a stage is assigned to it based on how advanced it is. The stage and site of breast cancer determine the appropriate treatment and the prognosis. There are several options for breast cancer treatments including surgery, radiotherapy, chemotherapy, and gene therapy (Cristofanilli et al., 2003, Akram et al., 2017).

Surgery

The first step and the most common treatment of breast cancer is surgery. It involves removing the tumor and the surrounding tissues that might be cancerous, known as

margin. There are two types of surgery in breast cancer treatment, which are removal of the lump only (lumpectomy) and surgical removal of the entire breast (mastectomy) depending on the stage and type of tumor. The goal during breast cancer surgery is to make sure the tissue removed has margins that are clear from tumor, indicating the cancer has been fully excised. Otherwise, further surgery to remove left over tissue may be required (Bellavance and Kesmodel, 2016, Akram et al., 2017).

Chemotherapy

Chemotherapy is the treatment of cancerous cells by using anti-cancer drugs. Chemotherapy is often given in cycles of treatment, recovery and treatment again. Selection of which patients are eligible for chemotherapy is based on overall health, age, medical history, type, stage of cancer and patient tolerance for specific medications. Chemotherapy can also be given before surgery to shrink the tumor and make breast conserving surgery possible compared to mastectomy (Lopez et al., 2008, Anampa et al., 2015).

Radiotherapy

Radiotherapy involves radiation, such as x-rays or gamma rays that target tumor directly. This treatment is usually performed after surgery and is very effective in killing cancer cells that remain after surgery. Usually, the dose of radiation used must be powerful enough to ensure the cancer cells can be eliminated. Treatment using radiotherapy is scheduled for up to five times a week for five to seven weeks (Goyal et al., 2010).

Gene therapy

Gene therapy is one of the most recent treatments involved in the management of breast cancer. Gene therapy is an experimental technique that uses gene(s) to treat or prevent disease. Based on the study of cancer arised due to the accumulation of genetic modification, gene therapy was considered as a newly potential therapy to inhibit cancer cells. This technique allows doctors to treat cancer by inserting a gene of interest into a patient's cells. There are several approaches applied by the researchers in gene therapy such as replacing a copy of the mutated gene with a healthy gene, inactivating a mutated gene and inserting a new gene to prevent or treat a disease in the body. The clinical studies of gene therapy on breast cancer were generally well-tolerated, with a few side effects reported. For example, treatments of transgene using a designed oncolytic viruses as a vector to the six patients with recurrent breast cancer decreased tumor size up to 30-100 % (Kimata et al., 2006, Love and Uy, 2008, McCrudden and McCarthy, 2014).

1.3 Cancer cell metabolism

Normal cells are equipped with complex and systematic signaling network that are coordinated by key control enzymes, operating metabolic machineries and sensing environmental signals to provide sufficient energy for survival. They are expected to grow, divide and die in a perfectly controlled manner (Jang et al., 2013). However, cancer cells exhibit different metabolic requirements and altered metabolic activities compared from those of normal cells. Altered energy metabolism is associated with increased metabolism of glucose, protein and lipid in cancer cells (Fadaka et al., 2017). These alterations in metabolic activities are needed to support the relentless cell division

and maintain the malignant properties of the cancer cells. Other than that, cancer cells undergo metabolic adaptation in order to promote their survival under conditions that kill normal cells (Cairns and Mak, 2016, DeBerardinis and Chandel, 2016).

Cancer cell metabolism is a direct effect of the modification of intracellular pathways that are disrupted either by mutated oncogenes and tumor-suppressor genes or both. In a condition where the deoxyribonucleic acid (DNA) or genes of a cell are mutated, and the damaged cannot be repaired, the cells are no longer able to control the normal cell growth. The mutated oncogenic genes can directly result in the development of cancer (Hanahan and Weinberg, 2000, Jang et al., 2013). Current genomic sequencing have shown that cancer cells exhibit a wide range of genetic changes that disrupt the normal pathways, including cell growth and DNA replication (Copeland and Jenkins, 2009, Hudson et al., 2010). The altered energy metabolism of cancer cells, including mitochondrial, lipid and glucose are generally very similar which are to meet the bioenergetics requirements, biosynthetic demands and balance redox conditions of the cancer cells (Cairns and Mak, 2016, DeBerardinis and Chandel, 2016). Glucose metabolism, which involved high glycolysis and lactate formation is marked as a key metabolism that associates with aggressive tumor phenotypes (Granchi et al., 2014).

1.3.1 Glucose metabolism in cancer cells

In normal mammalian cells, glucose is the primary source and main fuel for cellular respiration, with glycolysis as a universal pathway for the catabolism of glucose. Glycolysis is the series of biochemical reactions that break down glucose into pyruvate and generate waste product, lactic acid (Chaudhry and Varacallo, 2019). In the presence

of oxygen, the pyruvate will undergo oxidative phosphorylation inside mitochondria to produce energy in the form of high-energy phosphate compound known as adenosine triphosphate (ATP). Whereas in the absence of oxygen, the pyruvate is reduced to lactate before being transported out of the cells (Figure 1.3) (Gatenby and Gillies, 2004, Schurr, 2017). Under normal condition of glucose metabolism, about 70 % of ATP is synthesized by the oxidative phosphorylation (38 ATPs) and the rest by glycolysis (2 ATPs) (Fadaka et al., 2017).

Cancer cells require high amounts of energy in a short period of time for rapid proliferation and survival. Theoretically, cells undergo oxidative phosphorylation to generate high amounts of ATP rather than converting glucose into lactate which is far less efficient and produce less ATPs. However, cancer cells opt to generate ATP by glycolysis rather than oxidative phosphorylation (Hamanaka and Chandel, 2012). Most cancer cells exhibit high rate of aerobic glycolysis, which characterised as emerging hallmark in cancer metabolism (Xie et al., 2014b).

Aerobic glycolysis is different to the anaerobic glycolysis that converts the glucose to lactate only in the absence of oxygen (Dashty, 2013, Jeff and Matthew, 2018). Cancer cells exhibit high aerobic glycolysis regardless of oxygen availability, even in presence of ample oxygen. In cancer cells, this event is characterized by elevation of glycolysis and lactate production with increased of glucose uptake (Vander Heiden, 2011, Jiang, 2017).

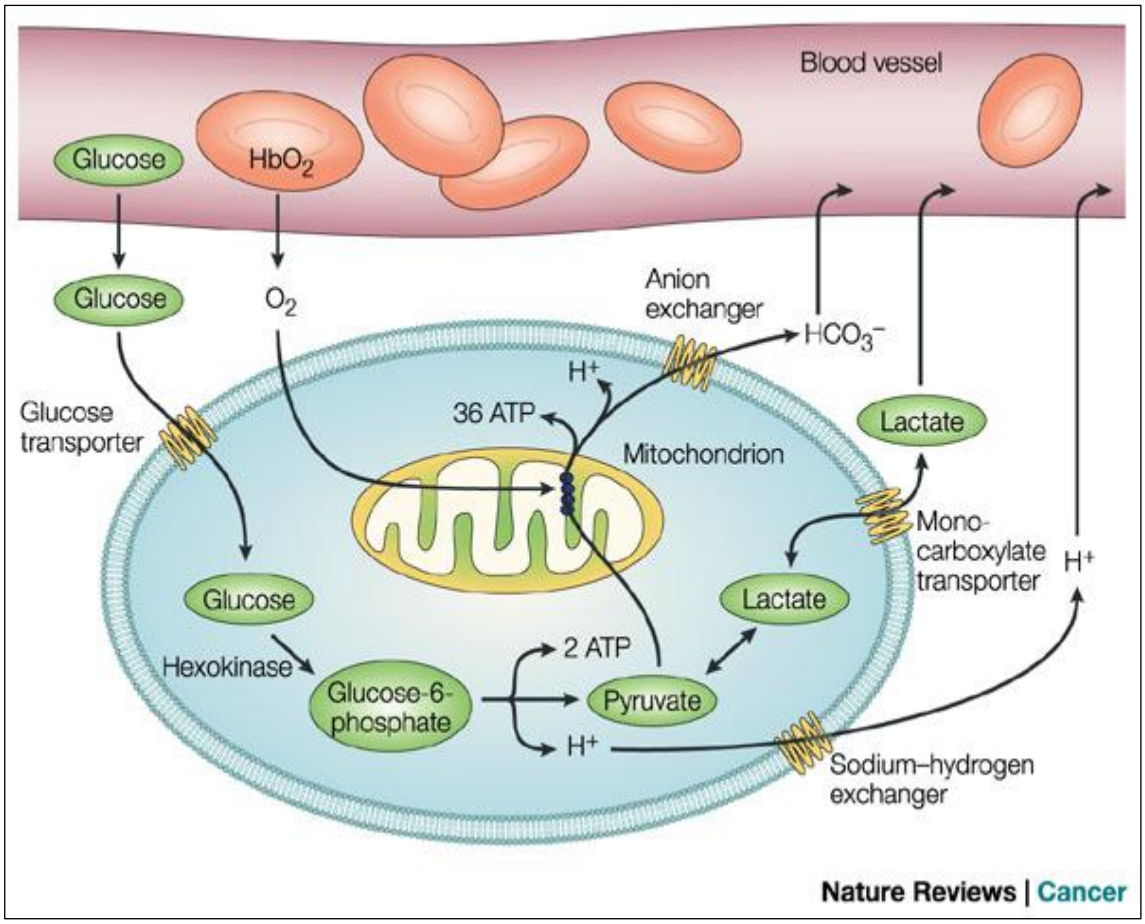

Figure 1.3 Glucose metabolisms in mammalian cells

The glucose enters the cells through glucose transporter (GLUT) and undergoes glycolysis process to produce pyruvate. Under aerobic conditions, pyruvate will enter the mitochondria to be oxidized to acetyl-CoA and produce 38 ATPs through oxidative phosphorylation. Under anaerobic conditions, pyruvate is reduced to lactate and excreted into extracellular space through monocarboxylate transporter (MCT). (Extracted from Gatenby and Gillies, 2004).

The phenomenon of high glycolysis in cancer cells can be visualized by using F-deoxyglucose positron emission tomography (FDG-PET) in tumors. FDG-PET imaging from thousands of oncology patients have shown significant increased glucose uptake and variable secretion of lactate in human cancers compared to normal cells (Kostakoglu et al., 2003, Zhu et al., 2011). FDG-PET imaging also showed that increased glucose uptake correlated with poor prognosis and tumor aggressiveness (Gatenby and Gillies, 2004, Caresia Aroztegui et al., 2017).

1.3.2 Consequences and implications of aerobic glycolysis in cancer

Increased glucose uptake in cancer cells may result from adaptive responses to a low oxygen level of tumor environment or known as hypoxia. During hypoxia, the oxidative phosphorylation will be inactive with mitochondrial dysfunction (Hu et al., 2012, Eales et al., 2016). Thus, cancer cells cannot obtain sufficient ATPs (Jang et al., 2013, Ganapathy-Kanniappan and Geschwind, 2013). Study by Otto Warburg observed that tumor cells continuously utilised glucose for glycolysis regardless of whether the cells are well-oxygenated or not (Koppenol et al., 2011). The breakdown of glucose to lactate produces only 2 ATP, but the production is faster compared to oxidative phosphorylation. The rate of glycolysis and conversion of glucose to lactate in cancer cells is accelerated may be 100 times faster, thereby resulting in rapid ATP production (Pfeiffer et al., 2001, Locasale and Cantley, 2011) and other consequences for cancer cells as simplified in Figure 1.4.

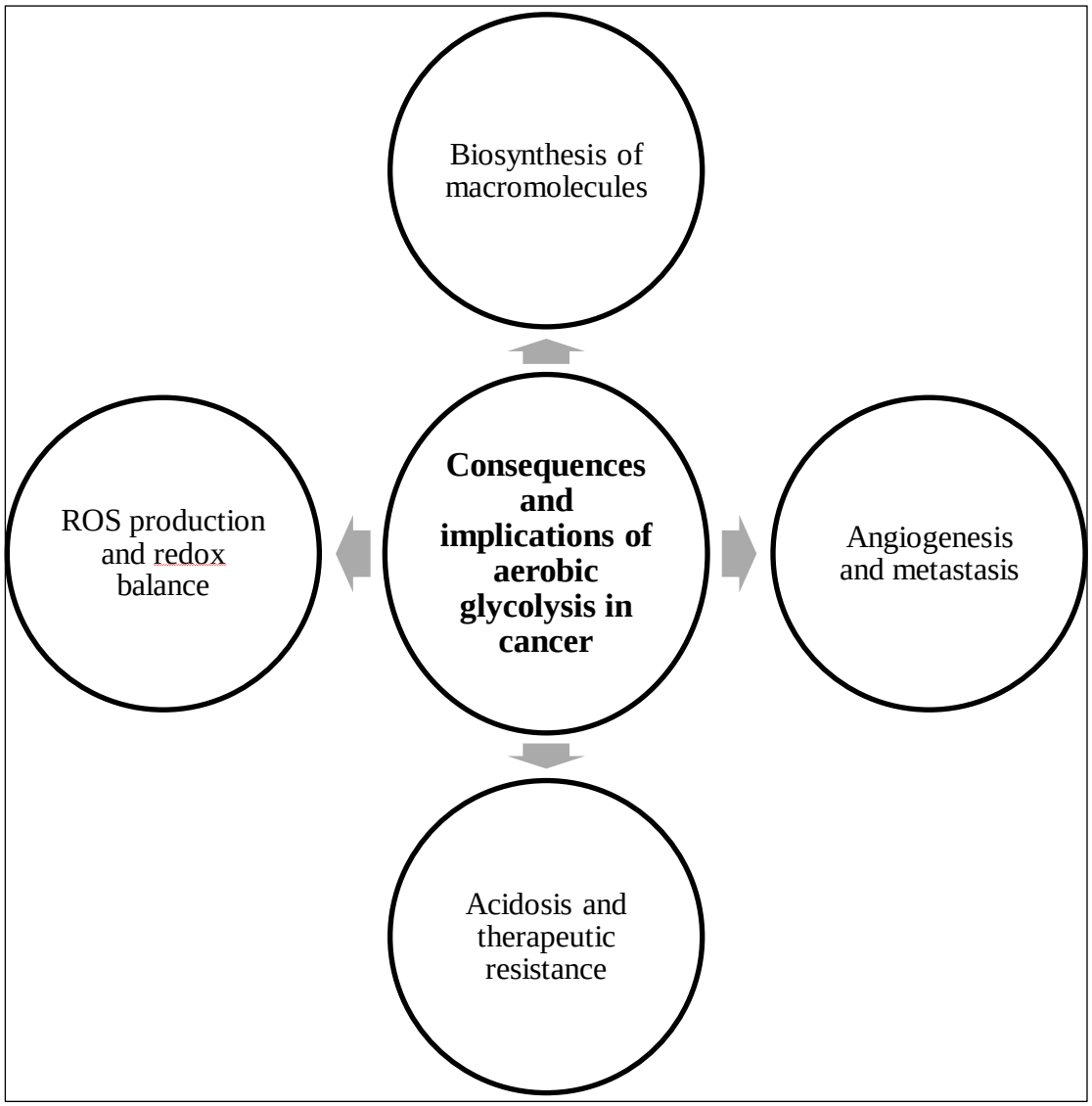

Figure 1.4 The consequences and implications of aerobic glycolysis in cancer cells.

Cancer cells tend to convert most glucose to lactate regardless of whether oxygen is present (aerobic glycolysis). Its benefits to the cancer cells in various ways, thus maintaining the cancer cell survival.

Biosynthesis of macromolecules

Increased glucose uptake in cancer cells is part of the metabolic adaptation for survival (Cairns and Mak, 2016). High glycolysis and increased glucose uptake may be beneficial to cancer cells through the production of glycolytic intermediates that will be used as precursors for many biosynthetic pathways. Biosynthetic pathways are essential aspect in cancer metabolism because they allow cells to generate macromolecules such as nucleotides, lipids, amino acids and NADPH, which are required for rapid cell proliferation and tumor growth (Lunt and Vander Heiden, 2011).

Angiogenesis and metastasis

Hypoxia condition of the cancer cells drives the development of pre-malignant lesions that grows progressively away from the blood supply and upregulates the angiogenic factors (Semenza, 2012). Angiogenesis is the process by which tumor develops new vessels for blood supply. Initially, tumors can obtain oxygen and nutrient by simple diffusion, but as they grow excessively, they need to develop new blood vessels to fulfill the rapid requirement for growth, invasion and metastasis. The hypoxic conditions in cancer cells resulted in the upregulation of hypoxia-inducible factor apha 1 (HIF1α) expression. HIF1α overexpression is strongly associated with promoting tumor growth and metastasis through its role in initiating angiogenesis and regulating cellular metabolism by activation of HIF1α target genes (Eales et al., 2016).

Acidosis and therapeutic resistance

High glycolysis rate in cancer cells results in increased lactate production, the-end product of glycolysis. Since the excess cytosolic acidification can inhibit the glycolysis

pathway of the cells, lactate is then excreted out from the cells through monocarboxylate transporters (MCT) (Bonen, 2001, Halestrap and Wilson, 2012). A constitutive production of lactate will generate an acute cytosolic acidification and lower the extracellular pH of the cells, approaching value 6.0. Studies have shown that under acidic environment, normal cells are unable to grow due to the lack of mechanism to adapt to the pH imbalance (Raghunand et al., 2003, Swietach, 2019). However, cancer cells are able to proliferate continuously regardless of imbalanced pH. The evolutionary potential and adaptation of the cells towards the acidosis accelerates the malignant progression and acquire a resistance to the therapeutic strategies. Moreover, the acidosis itself can be a mutagenic factor to the nearby normal cells (Gatenby and Gillies, 2004, Gharia et al., 2018).

ROS Production and redox balance

Reactive oxygen species (ROS) are heterogeneous compounds of highly reactive chemical species containing oxygen. ROS are produced as a natural by-product of many metabolic pathways such as oxidative phosphorylation, oxygen metabolism and NADP/NADPH oxidative functions. Due to their high reactivity characteristics, their effects are unspecific, that can be either beneficial or detrimental (Cairns et al., 2011). At high metabolic rates of cancer cells, the ROS production is increased, resulting a shift in the redox balance that can promote even higher growth rate and cancer progression. Thus, the accumulation of ROS for up to the toxic levels will induce severe oxidative stress and trigger the cell death or senescence (Reuter et al., 2010). As a defense mechanism to survive, cancer cells must up-regulate antioxidant mechanism that can scavenge the ROS (Anastasiou et al., 2011, Cairns et al., 2011).

In cancer cells, HIFs mediated the stimulation of glycolysis in order to inhibit further mitochondrial production of ROS, and activate reducing system that can scavenge these ROS. Increased glucose uptake is frequently observed during the conditions of oxidative stress. Studies showed that high ROS production induces glucose uptake, which suggested the role of glucose in ROS scavenging (Merry et al., 2010, Pinheiro et al., 2010, Andrisse et al., 2014).

The activation of pentose phosphate pathway (PPP) (a metabolic pathway parallel to the glycolysis) will generate NADPH to reduce the glutathione (GSH), which is an antioxidant that can detoxify the ROS. Since accumulation of ROS can shut down the cell metabolism pathway, the flow of glucose to PPP is critical for cancer survival to produce enough GSH in order to detoxify the excess ROS (Anastasiou et al., 2011).

1.3.3 Key players in glucose metabolism of cancer cells

Glucose transporters (GLUTs)

The first rate-limiting step of glucose metabolism is the transport of glucose across the plasma membrane mediated by facilitative glucose transporters (GLUTs) proteins. GLUTs permit the energy independent transport of glucose across the cell membrane (Macheda et al., 2005, Adekola et al., 2012). There are 14 members of the mammalian GLUTs family that have been identified and each of the members possesses different affinities for glucose or other hexoses. For example, GLUT1, GLUT3 and GLUT4 have high affinity for glucose and allowing transport of glucose at a high rate under normal physiological environment (Navale and Paranjape, 2016). Meanwhile, GLUT2 has high affinity and mediated fructose uptake in the cells (Barron et al., 2016).