Review Article

Triple negative breast cancer: The role of metabolic pathways

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Abstract

The incidence of breast cancer in Malaysia and other Asian countries is on the increase, reflecting lifestyle changes some of which are known risk factors for the development of breast cancer. Most breast cancers are amenable to adjuvant therapies that target hormone receptors or HER2 receptors on the surface of the cancer cells and bring about significant improvement in survival. However, approximately 17% of Malaysian women with breast cancer, present with tumours that are devoid of these receptors and are consequently termed ‘triple negative’ breast cancers. These triple negative breast cancers typically occur in women of a younger age than receptor positive cancers, are predominantly of high grade tumours and the prognosis is usually poor. There is therefore a pressing need to understand the biological pathways that drive these tumours, in order that effective strategies are developed to treat these aggressive tumours. With the increasing affluence of developing countries, obesity and Type II Diabetes are also on the rise. These diseases are associated with an increased risk of developing a range of cancers including those of the breast. In particular, the metabolic syndrome has been shown to be associated with triple negative breast cancer. This article reviews some of the metabolic pathways and biomarkers which have been shown to be aberrantly expressed in triple negative breast cancer and highlights some of the ongoing work in this area.

Keywords: triple negative breast cancer, basal-like, fatty acid binding protein, insulin-like growth factors.

Introduction

Breast cancer in Malaysia is on the increase, in line with most developing and medium income countries in Asia and it is the most common cancer in women. As countries become more affluent there is increasing ‘westernisation’ with respect to lifestyle. This term refers to multiple environmental influences, which are known risk factors for breast cancer to include; obesity and change to more dairy based diets, null or late parity and a reduction in breast feeding following childbirth. In Asia, women generally have less oestrogen exposure that women in the UK; they tend to start menstruating later and go through the menopause earlier; they tend to bear children younger and breast-feed for longer and the use of the contraceptive pill is very low.1

In the West approximately 80% of breast cancer cases occur in patients of 50 years or over whereas in Malaysia less than 50% occur in this age category and 60% of patients are pre-menopausal, whilst in the West the majority of breast cancers occur in post-menopausal women.2,3 There are also differences in the mean age of occurrence between the three main ethnic groups in Malaysia; in Malays it is 48.1 years, Chinese 51.4 years and Indians 52.3 years.2

There is a tendency for Malaysian women with breast cancer to present at a late stage, due to amongst other reasons; a lack of a screening programme to detect non-symptomatic tumours at an early stage, and cultural perceptions of cancer. With late stage, come a worse prognosis and a poorer survival rate compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the US. Due to the prevalence of the disease and the poor survival compared to women with breast cancer in Europe and the
growth factor receptor 2 (HER2) positive disease and will improve survival, particularly in those that present at an early stage. However, there remains approximately 15% percent of breast cancers that do not overexpress oestrogen receptors (OR), progesterone receptors (PR) or HER2 and therefore do not respond to these targeted therapies. Due to their lack of expression of these three markers, they have been termed ‘triple negative’ (TN) breast cancers and are the subject of this review.

Triple negative breast cancer

The incidence of TN breast cancer cases in Malaysia is around 17%, a rate similar to that found in Western studies.4 Triple-negative (TN) breast cancers tend to be of a higher grade and stage than other breast cancers; they also tend to have poorer survival and are more likely to metastasize to distant sites.6-8 Compared to other breast cancers TN breast cancers occur in a younger age group, for example a median age of diagnosis of 48 years was reported by one group, whilst another found that TN breast cancer was more common in those under the age of 40.8,9

TN breast cancers are less likely to be diagnosed by screening methods such as mammography or ultrasound; the reasons for this could be due to the rapid growth rate of TN tumours or differences in tumour density of TN breast cancers compared to other breast cancer types making their detection more difficult by imaging techniques.10 Currently, patients with these highly aggressive tumours have a poor prognosis, with no specific therapy available for their treatment unlike hormone receptor and HER2 positive breast cancer.11 To define TN breast cancers by markers they do not express is an unsatisfactory way of classifying and studying these important tumours. Fortuitously, expression profiling has identified a group of breast carcinomas that are to some extent synonymous with triple negative breast cancer, in that the majority of them are negative for OR, PR and HER2.

Basal-like breast cancer

Breast cancers have been traditionally defined by their histological features i.e. their appearance under the microscope when stained with haematoxylin and eosin or by the markers they express with immunohistochemistry (IHC). Molecular profiling techniques, such as expression arrays, are becoming more commonplace in defining breast cancer types. The different molecular subtypes of breast cancer reflect the expression of distinct gene patterns. The luminal subtypes of breast cancer tend to express hormone receptors and have properties of luminal epithelial cells such as expression of low molecular weight cytokeratins; ERBB2+ breast cancer over-express HER2 and the ‘normal breast cancer’ type were found to express genes associated with adipose tissue and non-epithelial cells such as lipoprotein lipase and integrin-α7.12,13 Basal-like breast cancer (BLBC) is so named due to expression of genes typical of basal/myoepithelial cells, such as high molecular weight cytokeratins.12-14 The consensus is that these types of breast cancer originate from the myoepithelial or basal layer of cells within the duct. Cytokeratins (CKs) are intermediate filament proteins that help comprise the mammalian cell cytoskeleton. The expression of particular CKs reflects the type, differentiation state and function of the epithelial cell. The expression of CKs can aid the classification of breast cancer;12,13,15-17 For example, luminal epithelial cells, and therefore breast cancers of luminal type, typically express higher levels of CKs such as CK7, CK8, CK18 and CK19; whilst myoepithelial or basal cells tend to express CK5/6, CK14 and CK17.14,15 However, there is a debate as to whether BLBC actually originate from the myoepithelial/basal cells or just share a similar phenotype.18 Since molecular profiling is not currently cost efficient and feasible to carry out on a large scale, in particular on routine surgical specimens, IHC staining of the basal CKs such as CK5/6 CK14 and CK17 has been found to be an effective alternative to identify BLBC.11,16,19 From molecular profiling and IHC studies there is an overlap between TN and BLBC; 56-84% of TN cases express basal markers however not all BLBCs are TN.11-14,20 BLBC, like TN breast cancers also tend to be associated with poorer survival. Rakha et al.,17 reported similar findings; BLBC was associated with the poorest survival rates in both lymph node negative and positive cases. The development of BLBC has been associated with certain risk factors; for example, early menopause, multi-parity and not breast feeding following childbirth; as with TN breast cancer, breast cancer at a younger age was also more likely to be BLBC.21
The role of metabolic pathways in breast cancer

Obesity is on the increase worldwide particularly in western countries and it is estimated that it will increase healthcare costs by around £2 billion by 2030 in the UK due to the increased risk of developing diseases such as diabetes, heart disease and cancer. Postmenopausal women with a body mass index (BMI) of over 30 have a 31% increased risk of developing breast cancer compared to postmenopausal women with a BMI below 25; obese women who develop breast cancer also tend to have poorer overall survival and increased breast cancer progression and metastasis than women of a healthy weight. One study reviewed the evidence for the association between obesity and breast cancer and proposed the involvement of several pathways. Increased fat levels or adiposity can increase circulating levels of insulin and insulin-like growth factor-I (IGF-I), tumour necrosis factor-α (TNF-α) and adipokines such as leptin. TNF-α is an inflammatory cytokine that is secreted by adipocytes that can lead to increased expression of aromatase, thus activating hormonal dependent tumorigenic pathways. Leptin is also secreted by adipocytes and regulates food intake by acting on the hypothalamus. Leptin can act as a growth factor in breast cancer cell lines, resulting in a more aggressive phenotype. Lorincz and Sukumar (2006) used the evidence to propose the mechanisms by which obesity is linked to breast cancer. However, as the majority of these mechanisms occur through the oestrogen receptor, this does not take into account the link between obesity and breast cancers that do not express oestrogen receptors such as TN breast cancers and many HER2 positive tumours.

Type 2 diabetes

Type 2 diabetes is associated with an increased breast cancer risk; insulin resistance leads to increased levels of circulating insulin that can activate signalling pathways such as RAS and PI3K, through the insulin receptor and cause increased proliferation and decreased apoptosis. There is some evidence linking other aspects of metabolism and TN breast cancer. For instance, the metabolic syndrome is a combination of medical disorders such as central obesity, raised triglyceride levels in the blood and high blood pressure, which substantially increase an individual’s risk of developing heart problems or diabetes. Studies have investigated the association of breast cancer with the metabolic syndrome and its associated diseases; metabolic syndrome is more common in patients with TN breast cancers than other breast cancers but it is yet to be determined whether the metabolic syndrome increases the risk of TN disease.

BRCA1

Breast cancers with BRCA1 abnormalities tend to be of the TN/basal like phenotype. In addition to its tumour suppressor gene function, operating through cell cycle controls, BRCA1 has also been shown to be involved in the regulation of fatty acid synthesis through interaction with acetyl-coA carboxylase (ACC). BRCA1 binds to the phosphorylated or inactive form of ACC, the rate limiting enzyme in fatty acid synthesis, and stabilises it. If BRCA1 is mutated or present in low levels, ACC is more abundant in its active form, thus converting acetyl coA to malonylcoA to increase fatty acid synthesis by fatty acid synthase. It has been suggested that women with BRCA1 mutations could be treated with diabetic drugs such as metformin or through calorie deprivation and exercise, as this would increase AMP-activated protein kinase (AMPK) levels that would in turn inhibit ACC. Fatty acid synthase (FAS) is up-regulated in many cancers including those of the breast, prostate and colon. FAS expression is associated with an increased risk of breast cancer reoccurrence and up-regulation of FAS confers chemo resistance; down regulation of FAS causing breast cancer cell lines to become more sensitive to chemotherapy drugs. Insulin-like growth factor (IGF) -I has been shown to up-regulate FAS in malignant breast cancer cells and when FAS was suppressed, IGF-I mediated cell growth was inhibited.

The Warburg effect

The Warburg effect describes the phenomenon by which cancer cells rely on aerobic glycolysis for energy rather than oxidative phosphorylation. The Reverse Warburg effect is based on the observation that the benign tissue, to include stromal fibroblasts, surrounding cancer cells also uses aerobic glycolysis for energy. It is postulated that epithelial cancer cells “stimulate” the fibroblasts to undertake aerobic glycolysis and thus secrete the products lactase and pyruvate. These metabolites would effectively “feed” the cancer cells resulting in increased proliferation. One study found that human breast cancer stromal tissue had features of the Reverse Warburg effect such as inflammation and markers of aerobic glycolysis. It suggested that loss of
Cav-1 may be a useful biomarker of oxidative stress in the tumour microenvironment. Others have found that markers associated with the Reverse Warburg effect predict a poor outcome in breast cancer patients. It has been suggested that an increase in reactive oxygen species may cause mutations in mitochondrial DNA and thus dysfunctional metabolism at the cellular level. In addition, a recent article highlighted a link between glucose metabolism, reactive oxygen species production and Epithelial-Mesenchymal Transition (EMT), a feature often associated with basal-like breast cancer.

**Fatty acid binding proteins**

Fatty acids in tissues are frequently bound to binding proteins, which are thought to play a role in their solubilisation, transport, storage and metabolism. There are 9 fatty acid binding proteins (FABPs), all around 15kDa in size with a β-barrel tertiary structure that forms a cavity where FAs bind; additionally FABPs have a helix-loop-helix motif which is thought to be the point of entry for the FAs.

**Fatty acid binding protein 7**

Fatty acid binding protein 7 (FABP7) is also known as brain lipid binding-protein (BLBP/FABP-B) as it was first discovered in the brain. Gene expression analysis shows Fatty acid binding protein 7 (FABP7) is frequently overexpressed in BLBC. There is conflicting evidence as to whether FABP7 expression (Fig 1) is associated with poor or good prognosis; one breast cancer study using IHC concluded that FABP7 was associated with improved survival probability; another concluded that FABP7 was associated with poor survival. Such differences could be a reflection of the cohorts being selected and differing in size, and also in how the results are interpreted. In vitro studies have shown that FABP7 inhibits cancer cell growth; overexpressing FABP7 in the MDA-MB-231 breast cancer cell line significantly inhibited cell growth and promoted differentiation. This effect was synergised with supplementation of the fatty acid docosahexaenoic acid (DHA).

In contrast a study on FABP7 in melanoma cell lines found that down-regulating FABP7 expression decreased cell proliferation and invasion and increased when FABP7 was over-expressed. When tested by IHC in patients with melanoma, the expression of FABP7 was associated with poorer survival and relapse, although FABP7 expression was lost when patients progressed from primary to metastatic disease. Loss of heterozygosity of the FABP7 gene in metastatic melanomas may be responsible for the loss of FABP7 expression and thus improved prognosis. In neural glioblastomas FABP7 is also associated with a poor prognosis and in animal models dietary supplementation of DHA has been found to increase FABP7 mRNA in the brain of rats.

FABP3, also called mammary derived growth inhibitor (MDGI), has high sequence homology with FABP7 and has been shown to be down-regulated by IGF-1 in mutant mice. Furthermore

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**FIG. 1:** A triple negative invasive ductal carcinoma (IDC) showing strong cytoplasmic and nuclear staining for FABP7, in the invasive tumour compartment.
it was found that insulin like growth factor-II (IGF-II), a ligand of the IGF axis, expression was inversely correlated with FABP3 expression. Nevo et al.\(^5\) found that one possible reason for the tumour suppressing activity of FABP3 is that it regulates integrin activity which in turn has a role in cell motility, growth and adhesion. Considering the sequence homology of FABP3 and FABP7 it may be possible that FABP7 also has interactions with integrin, IGF-I and –II and thus the IGF axis. This evidence suggests that the role of FABP7 may be different in different cancers and that expression may relate to cancer progression and stage. However, the association of FABP7 and prognosis in breast cancer is not understood; learning more about it will help our understanding as to what drives TNBC.

**Insulin-Like Growth Factors and their binding proteins**

The insulin-like growth factors –I and –II (IGF-I and IGF-II) are essential in foetal growth and growth during childhood; links with the growth hormone axis and epidemiological studies have shown that IGFs are important in determining height. Interestingly, there is some evidence to suggest that individuals who are taller are more at risk of developing cancer particularly of the breast or prostate. Moreover, circulating IGFs have been linked to the risk of developing breast cancer; for instance one study found that increased serum IGFs were linked to an increased risk of developing breast cancer in premenopausal women but not those who were postmenopausal. Members of the IGF family include a series of six Insulin-Like Growth Factor Binding Proteins (IGFBP) and a type 1 IGF receptor (IGF-IR) to which both IGF-I and IGF-II bind and which regulate the mitogenic and anti-apoptotic effects of the two ligands.

High dairy product consumption has been associated with high serum levels of insulin like growth factors –I and II (IGF-I and IGF-II). A study by Rinaldi et al.\(^5\) found that high IGF-I serum levels were associated with higher breast cancer risk particularly in younger women. In Chinese women high IGF-I serum levels were also found to be associated with increased breast cancer risk but only in premenopausal women. It is interesting to note that traditionally the consumption of dairy products has been significantly less in Malaysia than in Western countries.

Low serum IGFBP-3 levels have been associated with an increased risk of developing breast cancer in premenopausal women. Both IGF and IGFBP expression fluctuate throughout mammary gland development and lactation; altering the expression of members of the IGF axis impacts on mammary gland growth and differentiation. A study, investigating IGFBP2 expression in the tissues from a cohort of women with breast cancer, found that the more aggressive the breast cancer the higher the IGFBP-2 expression when compared to benign lesions. IGFBP-2 has also been found to be over expressed in anti-hormone resistant breast cancer cell lines. So et al.\(^6\) demonstrated that whilst IGFBP-2 expression was not of prognostic value in hormone receptor positive or HER2 positive breast cancers; in the hormone receptor negative cases IGFBP-2 expression was associated with poorer disease specific survival.

It was originally thought that the actions of IGFBP-2 and the other IGFBPs were dependent on IGFs but IGF independent actions have been identified. It has been found that IGFBP-2 is a novel regulator of the tumour suppressor Phosphatase and Tensin-homolog (PTEN), Perks et al.\(^6\) illustrated that the breast cancer cell line MCF-7 does not respond to high levels of IGF-II as a result of PTEN induction and that IGFBP-2 suppresses PTEN when it is not bound to IGF-II. Alternatively, PTEN may regulate the expression of IGFBP-2 as demonstrated by Levitt et al.\(^6\) in the U251 glioma cell line; where inducing PTEN expression decreased the expression of IGFBP-2.

A recent immunohistochemical study on a cohort of Malaysian women with triple negative breast cancers, found a significant association between PTEN loss in the tumours and levels of IGFBP2 expression (Fig. 2), supporting the in vitro studies of Perks et al.\(^6\) However, whilst there was a trend towards poorer prognosis in the group with high levels of IGFBP2 in their tumours, the differences were not significant, possibly due to the relatively small cohort size.

**Future perspectives**

The role of metabolic pathways in carcinogenesis has in recent years received renewed interest.
The initial work of Warburg in the 1950’s, for which he received the Nobel Prize, had remained largely forgotten due to the focus on genetic mutations in the DNA as being the underlying cause of the majority of cancers. However, there is renewed interest in the concept that metabolic factors have a pivotal role to play in carcinogenesis and the progression of cancers. This approach not only proposes an indirect role, in that metabolic pathways are aberrantly expressed as an effect of mutated genes, but also that aberrant metabolism itself, such as that seen in obesity and Type 2 Diabetes, can be a causative event in carcinogenesis, due to its pro-inflammatory effects on the cell. In this review, we have focused on just a handful of metabolic markers which are aberrantly expressed in triple negative breast cancer. Elucidating whether these and a myriad of other metabolic biomarkers are bystander events or indeed key players in the process of carcinogenesis and the progression of triple negative breast cancers is the subject of ongoing research.

REFERENCES


FIG. 2: A triple negative invasive ductal carcinoma showing loss of PTEN staining in the tumour cells (left) and strong cytoplasmic staining for insulin like growth factor binding protein-2 (IGFBP-2), in the invasive tumour compartment (right).
TRIPLE NEGATIVE BREAST CANCER


