

Breast cancer and protein biomarkers

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Abstract

Breast cancer is a healthcare concern of women worldwide. Despite procedures being available for diagnosis, prognosis and treatment of breast cancer, researchers are working intensively on the disease in order to improve the life quality of breast cancer patients. At present, there is no single treatment known to bring a definite cure for breast cancer. One of the possible solutions for combating breast cancer is through identification of reliable protein biomarkers that can be effectively used for early detection, prognosis and treatments of the cancer. Therefore, the task of identification of biomarkers for breast cancer has become the focus of many researchers worldwide.

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INTRODUCTION

Breast cancer is one of the leading causes of death among women^[1]. It accounts for approximately 40 000 deaths in the United States annually and still imposes a significant healthcare burden on women worldwide^[2].

Cancer is a disorder of cells caused by an unpredictable genetic disorder^[3] which leads to growth that is visible as a tumor^[4]. It is typically considered as a disease of aging because the chances of developing this disease increase with age^[5]. Cancer is characterized by uncontrolled cell proliferation, disruption of apoptosis, sustained angiogenesis and increased cell ability to invade other tissues and metastasize^[6,7]. Today, cancer is one of the main causes of global mortality.

A biomarker is a biochemical substrate that indicates a physiological state or development of a disease^[8]. It is usually a biological substance found in blood, tissue or other body fluids. Useful biomarkers should have a strong association with the outcome of the disease. Among the common clinical usage of biomarkers are the diagnosis, prognosis, treatment and indicators for the risk of developing the disease^[9]. In the context of the disease's treatment, a biomarker whose expression levels change according to treatment can be used to determine the progress of the treatment^[10]. The presence of a specific biomarker is often used as an indicator to predict the response of patients to therapy^[11]. Nevertheless, before a biomarker can serve its purpose, a thorough evaluation of its reliability needs to be validated.

CANCER BIOMARKERS

Cancer biomarkers can be classified as cellular and humoral markers. A cellular marker is associated with cancer cells and therefore it provides prognostic information for the patient from which a therapeutic plan suit best to the patient can be determined^[12,13]. On the other hand, a humoral cancer marker is characterized by its detection in body fluids. It is secreted either by the tumor or during tumor disintegration^[13]. A humoral cancer marker is useful in the early detection of cancer,

especially in asymptomatic people with high risk of developing cancer^[12].

In terms of the usage of biomarkers, cancer biomarkers can be divided into three main categories, namely diagnostic, prognostic and predictive biomarkers. A diagnostic marker is used to detect the presence of the disease. A prognostic marker is used after the establishment of the disease status to predict the course of the disease and its recurrence and can be used to indicate the aggressiveness of the tumor. A predictive marker is used to predict the likely response of a patient to a drug prior to a treatment so that patients are classified as “responder” or “nonresponder” by the presence or absence of the marker. Such a prediction is important in designing clinical drug trials to define the intended use of the drug^[14].

CURRENTLY AVAILABLE BREAST CANCER BIOMARKERS

There are a few potential serum based biomarkers for breast cancer. Nevertheless, only two of the serum based biomarkers were approved by the Food and Drug Administration (FDA) for monitoring and treatment of the advanced or recurrence breast cancer, MUC-1 (CA27.29 and CA15-3) and carcinoembryonic antigen^[15,16].

MUC-1 mucins belong to a protein family secreted by the luminal surface of glandular epithelia. The up-regulation of MUC-1 mucin was detected in breast cancer patients, particularly in patients' serum^[17,18]. Family members of *MUC-1*-gene include *MCA*, *BRMA*, *CA549*, *CA27.29* and *CA15-3*. Among which, CA15-3 is reliable in indicating the clinical course of patients with metastatic cancer. The serum level of CA15-3 corresponds to the tumor size. Although CA27.29 was found to be more sensitive than CA15-3, it was less specific than CA15-3^[19].

Carcinoembryonic antigen (CEA) is a glycoprotein presence in the serum of cancer patients and can be detected using radioimmunoassay or enzyme-linked immunosorbent assay^[20]. CEA does not serve as a good indicator for metastatic breast cancer as the CEA serum level was found elevated in only 15% to 68% of patients^[21]. In addition, a high false-positive rate was detected among the normal population, where a false positive rate ranging from 10% to 27% was reported^[20], limiting its clinical applications. The main clinical application of CEA is for gastrointestinal cancers, specifically in colorectal malignancy. It is a very useful marker for the early detection of liver metastasis in patients diagnosed with colorectal cancer^[22].

Another potential serum based marker is CA-125, a useful serum marker for monitoring ovarian cancer and predicting the patient's response to therapy. Nevertheless, CA-125 had insufficient sensitivity for diagnosis of the disease^[23]. In addition, serum CA-125 has been shown to be elevated in various forms of cancer, including ovarian, pancreatic, breast, colon, lung and endometrial carcinoma, making it less specific to any type of cancer^[24]. It is commonly agreed that CA-125 has a lack

of both sensitivity and specificity as a marker for early stage disease. However, its specificity can be improved by combining CA-125 with various forms of sonography^[14]. Changes in serum protein levels may also indicate the response of patients to chemotherapy in HER2-positive breast cancer patients, where a few serum based proteins, namely alpha-2-macroglobulin, complement 3, hemopexin and serum amyloid P, can be used as indicators for patients' positive response to chemotherapy^[25].

Prediction of patients' response to drugs is important for effective cancer treatment. Recently, many predictive biomarkers have been suggested for different types of drug treatment. Estrogen receptor (ER) and progesterone receptor are useful indicators for prediction of breast cancer patients' responses to hormonal therapy in an adjuvant setting and metastatic disease. Heat shock protein 70 showed significant positive correlation for the usage of aromatase inhibitors in hormonal therapy^[26]. As for ER-positive breast cancer, truncated BH3 interacting domain death agonist^[27] and 14-3-3 proteins, including theta/tau, gamma, epsilon, beta/alpha and seta/delta isoforms, can be used as indicators for patients' responses to neoadjuvant chemotherapy^[28]. In the event of triple-negative breast cancer, defined by lack of expression of estrogen, progesterone and human epidermal growth factor receptor 2 (HER-2), heat shock protein 90 has been identified as a critical target for its treatment^[29]. On the other hand, FKBP4 and S100A9 were suggested as putative prediction markers in discriminating doxorubicin and docetaxel drug sensitive patients from drug resistant patients^[30], while Hsp27 was said to indicate doxorubicin resistance^[31]. The response of patients to neoadjuvant paclitaxel/radiation treatment can be evaluated by the overexpression of alpha-defensins and microtubule associated protein 2 to indicate a pathological complete response. In addition, the presence of alpha-defensins and MAP2 were also used to indicate patients' sensitivity to taxane-based treatment^[32].

Prognostic biomarkers provide information regarding outcome irrespective of therapy. Amongst the potential prognostic biomarkers for breast cancer are Ki-67 for a primary tumor, cyclins, urokinase plasminogen activator, p53, p21, pro- and anti-apoptotic factors, BRCA1 and BRCA2^[33-35].

The discovery of the HER-2/neu oncogene has led to the formulation of an anti-cancer drug for patients with breast cancer^[36]. HER-2 belongs to the type I transmembrane tyrosine kinase receptor family. HER-2 is an important regulator for cell growth, differentiation during embryogenesis and for mammary development during puberty. Deregulation of HER-2 signaling in mammary cells promotes breast tumorigenesis^[37]. Approximately 25% to 30% of human metastatic breast cancers over-expressed HER-2^[38]. Overexpression of HER-2 is a significant predictor of reduced survival and shorter time to relapse^[39,40]. This is because these tumors tend to grow faster and are more likely to metastasize than tumors that do not overexpress HER-2. Thus, HER-2 has become an important therapeutic target for

this subtype of breast cancer. When breast cancer is diagnosed, HER-2 status is routinely assessed by either immunohistochemical analysis for HER-2 expression or fluorescent *in situ* hybridization analysis for *HER-2* gene copy^[41]. Two HER-2-targeted therapies are approved by the United States FDA for treatment of HER2-overexpressing metastatic breast cancer, namely Trastuzumab (Herceptin) and Lapatinib (Tykerb). The presence of HER-2 overexpression is a predictive factor that may indicate successful use of trastuzumab (Herceptin)^[40]. Trastuzumab (Herceptin) is a recombinant humanized monoclonal antibody targeted against the extracellular HER-2 receptor. The initial clinical trials of trastuzumab to be used as a sole agent in HER-2-overexpressing metastatic breast cancer demonstrated a response rate ranging from 12% to 34% for a median duration of 9 months^[42].

Early detection of breast cancer increases the survival rate of patients^[43]. However, early symptoms of breast cancer are sometimes absent or not recognized. It is often detected in the advanced stage and is untreatable when the cancer is diagnosed^[16]. Thus, a reliable biomarker is needed to rule out breast cancer in the early state. Unfortunately, currently available tumor markers lack the specificity and sensitivity to be used in early detection of breast cancer^[13,16].

PROTEOMIC APPROACH FOR BIOMARKER IDENTIFICATION

Protein profiling studies on different types of cancer have been carried out by researchers throughout the world. The 2D-PAGE coupled with mass spectrometry, isotope coded affinity tags, multidimensional protein identification technology, protein array technology and surface enhanced laser-desorption ionization-time of flight are among the common technologies applied in proteomic study^[44-46]. The proteome of a cell contains all of the gene products that represent the functional output of the cell^[47]. This makes proteomics a promising tool for characterizing cells and tissues of interest and for biomarker discovery^[48].

It has been estimated that only 2% of human diseases result from single gene defects. As for the remaining 98% of human diseases, epigenetic and environment factors need to be considered as they affect both etiology and severity of the disease^[49]. Therefore, cancer biomarker discovery targeting protein expressions has become popular as proteomic approaches characterize both modified and unmodified proteins involved in cancer progression. In recent years, the emerging sciences of genomics and proteomics have revealed the identity of proteins that can potentially serve as cancer biomarkers. Unfortunately, very few of these biomarkers are reliable clinically for prognosis or diagnosis of the disease and even fewer have been validated and approved for clinical usage^[50,51].

The proteomics of normal breast and cancerous tissue was first reported by Wulfschlegel *et al*^[52]. The authors

revealed 57 differentially expressed proteins between normal and cancerous tissues, including VDAC, transgelin, Hsp 27, GRP78 and cathepsin. Following this study, Somari *et al*^[45] reported the identification of annexin V, HSP 90, carbonic dehydrase, protein disulfide isomerase, gelsolin and fibrinogen beta chain. Luo *et al*^[53] added to the list of differentially expressed proteins, including manganese SOD, biliverdin reductase B, carbonic anhydrase I and annexin I, binding protein 4, cofilin 1, profiling 1 and uracil DNA glycosylase. On the other hand, Deng *et al*^[54] reported the potential of alpha-1-antitrypsin, EF-1-beta, cathepsin D, translationally controlled tumour protein, SMT3A and PSMA1 as candidate biomarkers for patients with breast cancer. Besides these studies, there are many recent reports on the identification of biomarkers in breast cancer^[26-30]. Although the potential use of most reported biomarkers for breast cancer has been scientifically proven, they need to be clinically validated.

HETEROGENEITY BETWEEN PATIENTS

It is a challenge to identify a common biomarker that fits all patients. Many different factors are involved in the development of cancer. They include age, race, family history, personal history of cancer, presence of viruses, mutations in cell regulation genes and tumor suppressing genes, exposure to carcinogens, lack of physical activity and diet^[55,56]. All these factors will cause heterogeneity in protein expression between patients. Therefore, individualized medicine has become a popular trend for treating cancer in Western countries. Nevertheless, in developing countries, such a treatment will be too expensive for most patients. In the author's laboratory, we have received surgically removed tissues from patients with breast and colon cancer. In general, Malaysia is a country populated by three main ethnic groups, namely Malays, Chinese and Indian. Although the environment and lifestyle factors are relatively similar between the ethnic groups, they originated from different parts of the world, with the Malays and Chinese mainly from China-Mongolia regions and the Indians mainly from south India. We have shown in our studies^[47] that the Malays and Chinese have relatively similar protein profiles, while Indians are very different. Therefore, the usability of each protein biomarker to be commonly used for a cancer per se among Malaysian patients was not reliable, but when an ethnic-specific biomarker was identified for each ethnic group, their significance greatly increased. Consequently, we have suggested the possibility of ethnic specific drug-targeted therapy, which may be more affordable to patients from developing or under developed countries where health insurance is not well established.

CHALLENGES FOR IDENTIFICATION OF BIOMARKERS FOR CANCER

A few criteria are required for biomarkers to be effec-

tively used in diagnosis, prognosis or treatment of cancer. First is the specificity of the biomarker to a type of cancer and such biomarker should be uniquely expressed in cancerous tissues only. Furthermore, consistent expression of the protein biomarker in cancer patients will surely increase its reliability as an indicator of the disease. Moreover, the abundant expression of the protein biomarker will ease its detection or its recognition as a target for treatment.

In the author's experience of researching the potential biomarkers for cancer, none of the proteins met the criteria of biomarkers in terms of consistent and unique expression in all cancer patients. Instead, we have detected many common proteins between normal and cancerous tissues that were differentially expressed^[57], indicating that the similar types of cell activities were operating between normal and cancerous cells while the rate of operation differs between the two tissues; cancer cell activities repeat more frequently than normal cells, which causes a rapid cell growth that leads to tumor formation. There may be uniquely expressed protein in cancerous tissue but such proteins may express themselves in low quantities, making them not a suitable biomarker for drug-targeted therapy.

CHALLENGES FOR IDENTIFICATION OF BIOMARKERS FOR DRUG-TARGETED THERAPY IN CANCER TREATMENT

Currently, most of the drugs used in chemotherapy are non-targeted, leading to two consequences. Firstly, the quantity of drug used in treatment was generally more than that required to fight cancer because the drug will be circulated in the body without a precise target, leading to the second consequence, the side-effects of the drug on patients who undergo chemotherapy treatment. The solution to this problem is drug-targeted therapy, where the drug will be targeted at the side of tumor so that the amount of drug used can be reduced and therefore the side-effect of chemo treatment can also be minimized. The key answer for drug-targeted therapy is the target itself, which should recognize the tumor and direct the drug to itself. Such a target must not be hidden and, at the same time, it must be easily accessible to drugs.

To date, there are many reports on the potential biomarkers for various types of cancer. Nevertheless, many projects seem to stop at the biomarker identification stage, which may be due to lack of funding or insufficient facilities to carry out the subsequent research. Moreover, innovation of targeted drug therapy is a lengthy process which could possibly take many years of continual research. Multi-factorial aspects on the safety use of the drugs also need to be examined. Such innovation requires large funding, while the revenue is hardly guaranteed as many of the drugs are eliminated even before reaching the second or third stage of a clinical trial. In drug targeted therapy, the drug of interest is

to be tagged on a specific antibody that recognizes the biomarker on the cancer cell. In order to be used as a biomarker in drug targeted therapy, the biomarker is best to be uniquely expressed on the tumor surface at high abundant quantity for easy recognition and access of the drugs. Although such biomarkers may not exist, there are abundantly expressed common proteins on the tumor surface that are differentially expressed, where their expression levels were found much higher in cancerous tissues than normal tissues. These differentially expressed abundant proteins on the surface of cancerous tissues may serve as a good target for recognition when its abundance is much higher than that of the normal tissues.

POSSIBLE SOLUTION BY COLLECTIVE USE OF BIOMARKERS

The use of an individual protein as the sole biomarker for diagnosis, prognosis or treatment for cancer no doubt simplifies the procedure. However, it has been shown in many studies that protein expression variation between patients is the main halting factor of the development of devices as the cost of such development is tremendously high; however, its usability is limited to a small group of patients. One of the possible solutions is the collective use of biomarkers to achieve the desired goal. Through principle component analysis and linear discriminant analysis statistical analysis, we have shown previously^[58] that the collective use of biomarkers in cancer has tremendously increased the correct identification of cancerous tissues. The combined use of biomarkers has also been shown to give a better prognosis and increase sensitivity and specificity to predict the response of patients to chemotherapeutic agents^[53]. This will also reduce the possibility of a false diagnosis as the reliability of a group of biomarkers is better than the sole use of a single biomarker.

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