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BIOTECHNOLOGICAL APPROACH TOWARDS BREAST CANCER

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ABSTRACT

Breast cancer is an ancient disease which was discovered 3500 years ago in Egypt. Breast cancer is the common malignant tumor and second cause of death in women all over the world. It is heterogeneity, including intra tumor (genomic, transcriptomic) and inter tumor (angiogenesis, metastatic) heterogeneity, Therefore no single treatment is sufficient to cure all patients. The Development of Molecular Profiling using DNA Microarrays, proved this heterogeneity. To determine the best therapies for Breast cancer patients, many factors have involved classifying the Breast cancer such as: age, Lymph node status, hormone receptor and etc. Based on current research techniques like Gene expression profiling, Immunohistology, Breast cancer identified into four types: Estrogen and Progesterone Receptor Positive, Luminal A and Luminal B, HER2 and Basal Like, Triple Negative. Each type has different prognosis and treatment response. Most therapies for Breast Cancer are: Surgery, Radiation, and Chemotherapy. Current research in cancer includes the development of carriers to allow alternative dosing routes, new therapeutic Methods such as Hormonal Therapy and Targeted Therapy and Proteomics.

KEY WORDS: **IHC, BRCA, FISH, Mutagen, HER2, DNA Microarray.**

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INTRODUCTION

Breast cancer starts when healthy cells in the breast change and grow uncontrollably, forming a mass or sheet of cells called a tumor. A tumor can be cancerous or benign. A cancerous tumor is malignant, meaning it can grow and spread to other parts of the body through the blood vessels and/or lymph vessels. A benign tumor means the tumor can grow but will not spread. There are several types of breast cancer. It can be diagnosed at different stages and can grow at different rates. This means that people can have different treatments, depending on what can work best for them. Breast cancer is the most common malignancy of women and is a disease of advancing age. Breast cancer is not one single disease, it is multifactorial disease. The biggest risk factors for developing breast cancer are getting older, being female and having a significant family history of the disease. Most cases of breast cancer happen by chance [2]. Only around 5% of breast cancers are caused by inheriting some altered genes. Treatment depends on the types of the cancer. For localized breast cancer, the most extent option is surgery to remove the breast, followed by radiotherapy. For tumors at greater risk of recurrence i.e. bigger, more aggressive cancers or cancers that have spread to the lymph nodes, additional treatment (adjuvant therapy) can be given after surgery. This can include hormone therapy of aromatase inhibitors or Tamoxifen for women whose tumors have Estrogen positive and/or Progesterone positive Breast cancer, and chemotherapy and targeted therapies such as Trastuzumab for those 25% of tumors that are HER2 positive (i.e. have the target for Trastuzumab on their surfaces).In last century, we have showed many progresses in medical and healthcare science, because of increasing life expectancy so, there are more opportunity for getting cancer in old age .Modern life is accompanied by various habits like processed food, motionless life style, smoking and drinking[3].

Molecular Testing of Breast Tumor

1. Estrogen receptors (ER) and progesterone receptors (PR) testing

Estrogen receptors (ER) and progesterone receptors (PR) may be found in breast cancer cells. Cancer cells that need estrogen or progesterone to grow and develop tumor are called (ER+) or (PR+) cancers. Use of hormone therapy drugs in such cases which reduce hormone levels or block hormone receptor can be useful for cancer treatment[8]. If the breast cancer cells do not have estrogen or progesterone receptors, treatment with hormone therapy is not helpful. Testing of the tumor for both estrogen and progesterone immunohistochemistry or IHC. IHC testing can detect estrogen and progesterone receptors in cancer receptors are a standard part of a breast cancer diagnosis. The most common method currently called cells from a sample of tissue[9].

2. HER2 testing

HER2 (human epidermal growth factor receptor 2) is one such gene that can play a role in the development of breast cancer. The HER2 gene makes HER2 proteins. HER2 proteins are receptors on breast cells. Normally, HER2 receptors help control how a healthy breast cell grows, divides, and repairs itself. But in breast cancers, the HER2 gene doesn't work correctly and makes too many copies of it. Extra HER2 genes make breast cells grow and divide in an uncontrolled way[10].Breast cancers with overexpression of HER-2 are called HER2-positive

- ***IHC test (Immunohistochemistry)***

The Immunohistochemistry test finds out if there is too much HER2 protein in the cancer cells with the help of adding special antibodies to sample which can cause color change if the number of HER-2 copies are high in the sample[11].

- ***FISH test (Fluorescence in Situ Hybridization)***

The Fluorescence In Situ Hybridization uses fluorescent pieces of DNA that only attached to copies of the *HER-2* gene in cells and can be counted under a special microscope. Modified technique, known as *chromogenic in situ hybridization (CISH)* works similarly to FISH by using small DNA probes to count the number of *HER-2* genes in breast cancer cells. The results of the FISH test can be positive or negative.

Gene Profiling

Several methods have been developed to look at hundred or more gene messages simultaneously. There are several distinct profiling platforms: (1) microarrays, (2) oligonucleotide Arrays, (3) complimentary DNA (cDNA) arrays and (4) multiplex RT-PCR[13]. The most applied of Gene Expression Profiling technique is arranged series of microscopic spots known as *probes* are covalently attached to a solid surface such as glass slide, a fibrous mesh membrane, forming a gene chip[12]. RNA is harvested from sample, labeled with fluorescent dye and hybridized to cDNA sequences on the gene chip and scanned for fluorescent labels at each spot. The level of fluorescence at a specific spot shows quantitative information about the expression of the particular gene, and then level of expression of each sample compared to the control, is collected and analyzed using statistical techniques. So, Gene profiling is used to identify specific alleles that grow risk of developing cancer in their lifetime[14].

Genome Analysis

This technique is used to study gene expression of one gene at a time, using PCR technology. Primers specific to the gene of interest were used in the PCR mix to find out whether that gene was being expressed in the tissue. Microarrays are used to compare the expression levels of thousands of genes all at the same time, enabling scientists to study a genetic profile. By using the microarray technology can identify which genes are turned on or off and also level of their expression[14]. Microarray analysis can help to identify genes that have a role in cancer. Real Time PCR technology is used to measure the gene expression in cancer cell line. This information as will use to identifies new targets for cancer therapy.

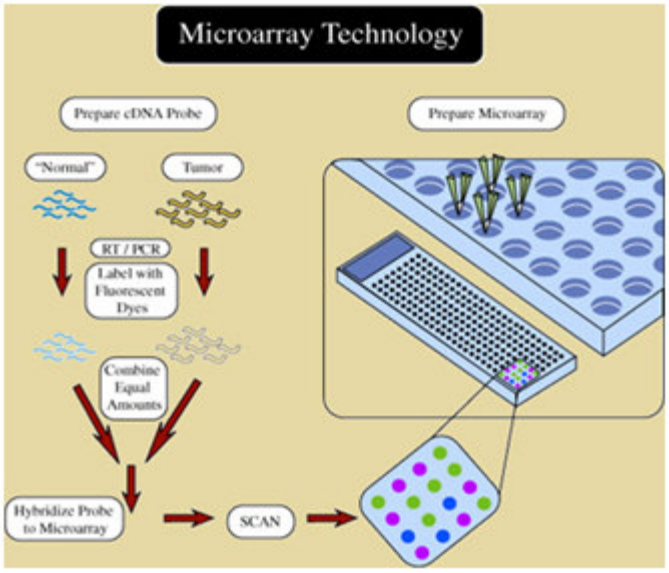

Figure1

Isolate mRNA from normal and tumor tissue samples, labeled cDNA with florescent Dye, after combination equal amount of both samples, make hybridization in Microarray then scan microarray for fluorescent

Cell Culture

Studies with cancer cell lines have played an important role in understanding of tumor biology and high throughput screening for drug development. However, accumulation of genetic aberrations of cancer cell lines that occur with increasing passage numbers have limited their clinical correction[14].Genetically altered cancer cell lines under *in vitro* condition do not truly represent clinical scenarios. First, the gene is cloned and introduced into a plasmid vector and then amplified in bacteria. The vector is then transferred into mammalian cells by cell transfection technologies such as liposomes. These cells are incubated at human body temperatures with essential nutrients. By comparing the gene expression in these cells with control cells it is possible to determine the change in cells caused by the inserted gene[5].

RECENT ADVANCED THERAPY IN CANCER

There are number of advances in cancer therapy as a result of new technologies such as Hormonal therapy, targeted therapies and combinational therapies. In addition, individualized therapies hold great potential for the future.

Hormonal therapy

Hormonal therapy is used as an adjuvant therapy to reduce the risk of the cancer recurrence after surgery. Ovaries are the main source of the hormone Estrogen till menopause[15]. After menopause, small amount of this hormone is made in the fat tissue, where a hormone made by the adrenal gland is converted into estrogen. Breast Cancer is a hormonally dependent disease in some patients. Estrogen and Progesterone promote the growth of cancer (ER+, PR+) [18]. Hormone therapy either reduces ER level or block ER to bind to its receptor in the surface of cancer cell. There are some drugs that block ER such as Tamoxifen and stop cancer cell growth and division. While Tamoxifen can act as anti-ER in tumor cell, it can acts as an ER in other tissue like uterus. Therefore, it is called a selective receptor modulator or SERM.Hormonal therapy is good treatment method for ER positive Breast cancer (Luminal A, Luminal B) [4].Basal Like / Triple Negative defined as tumors that are negative for ER, PR, HER-2 and is aggressive disease with limited treatment options, Due to lack of these receptors[17].

Targeted therapies

A targeted therapy is a technique to attack a certain molecular agent or pathway participated in the development of cancer. For example, the drug trastuzumab (Herceptin) is an antibody specific for Her2 which bind to receptor and block its function. The cell is no longer getting the influence to divide and grow rapidly and will stop growing and die[21].

Targeted therapies are drugs that block the growth and spread of cancer by interfering with specific DNA or protein molecules involved in cancer. Tumor-activated pro-drug therapy uses the drug conjugated to a tumor-specific molecule to remain inactive until it reaches the tumor. These systems would ideally be dependent on interactions between specific cells on the surface of cancerous cells and not the surface of healthy cells[20]. Limitations also exist due to the lower ability of some drugs after attaching to target when drugs are linked to portion of target incorrectly.

Proteomics

The foundation for any biomarker discovery is based on identification of proteins that show differential expression between disease and control samples. In general, there are two approaches to proteomic biomarker discovery: target specific and global/non directed. Target-specific approaches often use antibodies to screen specific proteins through western blot analysis, enzyme -linked immunosorbent assays, and antibody arrays[10].While these techniques are clinically applicable, they are generally low-throughput with regard to the number of proteins that can be

surveyed at any one time. Thus, they may not be ideal for biomarker discovery. In contrast, global/non-directed approaches may be better suited for biomarker discovery because they are relatively unbiased, high-throughput screens. Non-directed approaches can also be divided into two groups: Profiling of unidentified proteins and identified proteins[14].

Following are protein biomarkers used in breast cancer

Table 1
Candidate protein biomarkers in breast cancer

Biomarkers	Role
HER2 Proto oncogene	Development of cardiac and neural tissue
MMP-2 Protease	Digest ECM, OSTEOPROTEIN immune function
PAI-1	Regulation of plasmin formation
Cathepsin b	Functions in protein tumor sigma cell cycle progression
P53	Apoptosis
Cycline E	Cell proliferation

There are several identified candidate biomarkers, but these must be validated to prove their specificity and clinical relevance. One of these biomarkers is HER2; it is a member of the human epidermal growth factor receptor family. Amplification or overexpression of this oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer. In recent years the protein has become an important biomarker and target of therapy for approximately 30% of Breast cancer patients. MMP-2 and several other MMPs have been shown to proteolytically activate TGF-β, which has been shown to promote epithelial mesenchyme transition (EMT), a key process involved in cancer metastasis. Additionally, tumor overexpression of MMPs can be used to potentially target the release of chemotherapeutic agents specifically to tumor sites[23]. PAI-1 is among the best validated prognostic biomarkers currently available for lymph node-negative breast cancer, their main utility being the identification of lymph node-negative patients who have HER-2-negative tumors and who can be safely spared the toxicity and costs of adjuvant chemotherapy. Molecular suppression and selective therapeutic inhibition of cathepsin B significantly reduces pulmonary and bone metastasis. This study provides evidence that cathepsin B is a potential therapeutic target for treatment of breast cancer patients with metastatic disease. Over-expression of cyclin E correlates with tumorigenesis. Deregulation of cyclin E occurs in 18-22% of the breast cancers. Cyclin E is a prognostic marker in breast cancer, its altered expression increased with the increasing stage and grade of the tumor. Low molecular weight cyclin E isoforms have been shown to be of great pathogenic and prognostic importance for breast cancer. They are proved to be a remarkable marker of the prognosis of early-stage-node negative breast cancer[24]. The goal of the proteomic and genomic assays should be to develop biomarkers for screening, diagnosis, prognosis, and treatment monitoring.Together with genomics, proteomics is well on the way to molecularly characterizing the different types of biomarkers in breast tumor and thus defining new therapeutic targets for future treatment, as well as proteomics may be easily coupled with functional tests that are proximally impossible with genomic[31].

Gene therapy

One strategy for cancer gene therapy is that of genetic correction. Gene therapy implies an approach that aims to modify, delete or replace abnormal gene(s) at a target cell.A number of genetic correction strategies have been designed to treat cancer, such as correction of *p53* and *BRCA1*.*P53* is the most commonly mutated tumor suppressor gene in solid tumors and is also mutated in the germ line of patients with the rare hereditary Li-Fraumeni syndrome. The *p53* gene is directly related to progression of breast cancer development; because *p53* is frequently mutated in breast cancer and Li-Fraumeni syndrome. The function of unmutated p53 is suppression of cell proliferation through a multi protein regulatory pathway that is focused on control of apoptosis.*p53*may natural function is inhibition of cell proliferation; it inhibits cell growth in most normal and malignant cells26. Although identification of BRCA1 and BRCA2 genes make changes in treatment of patients with inherited Breast Cancer. BRCA1 and BRCA2 are involved in two molecular functions includes DNA damage repair or transcriptional regulation. Chromosomal instability as a result of BRCA genes deficiency can be basis of Breast Cancer formation. Over-expression of *BRCA1* into sporadic breast or ovarian cancer cells, which usually show low *BRCA1*expression, results in growth inhibition and tumor suppression[25]. *BRCA1* Cells with somatic mutation will be unable to repair DNA, so cells sustain DNA damage in many sites. For *BCRA1* tumor genesis, one of key checkpoint is p53, mutation in *p53* would inactive cell cycle checkpoint and lead to uncontrolled proliferation and invasive growth.Studies of adenovirus-based *p53* gene therapy and *BRCA1* retroviral vector gene therapy for cancer in both cell culture and animal models have demonstrated tumor suppression. Clinical trials of *p53and BRCA1* gene therapies for cancer have been reported and these studies demonstrate gene transfer of adenoviral-p53and retroviral –*BRCA1* induction of apoptosis and some indication of therapeutic response[25].The clinical application of gene correction therapy will require advances in both basic science and clinical research.Gene therapy has a synergistic effect when combined with chemotherapy, with higher tumor responses and lower therapy-related toxicities. Gene therapy is more successful in patients with earlier stages of malignancies or in those with lower tumor burden. Key problems at present include

the degradation of vector by the immune system and a need for higher levels of gene transduction. It needs vectors and vector delivery systems improvement[27].

CONCLUSION

Making anticancer treatment to specific populations of patients has become a reality thanks to success in building on experience acquired and to the fast development of understanding of tumor biology. The future of breast cancer treatment certainly depends upon ability to identify which therapeutic approaches is effective for which type of the breast cancer patients, using either predictive or prognostic indicators, or focusing on newly identified molecular targets[28]. Current research areas include development of carriers to allow alternative dosing routes, new therapeutic methods such as Targeted therapy, Gene therapy, Pharmaco-proteomics[30]. The degree of change in the quality of life and eventual life expectancy is directly related to this targeting ability of the treatments[29]. By combining proteomics data with bioinformatics tools can find significant protein-protein interactions which may contribute to the sequence of disease, response to treatment or gain of resistance[3]. This will transform the future of cancer therapy from generalized cancer treatment strategies, based on tumor size, nature and location to a more tailored, based on the specific genomic constituents, host immune status and genetic profile of the underlying malignancy. Treatment is expected to be fast, effective, relatively less toxic and inexpensive with higher cure rates[30].

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