MINI-REVIEW

Breast Cancer: Major Risk Factors and Recent Developments in Treatment

Wafa Majeed, Bilal Aslam*, Ijaz Javed, Tanweer Khaliq, Faqir Muhammad, Asghar Ali, Ahmad Raza

Abstract

Breast cancer is the most common in women worldwide, with some 5-10% of all cases due to inherited mutations of BRCA1 and BRCA2 genes. Obesity, hormone therapy and use of alcohol are possible causes and over-expression of leptin in adipose tissue may also play a role. Normally surgery, radiation therapy and chemotherapy allow a good prognosis where screening measures are in place. New hope in treatment measures include adjuvant therapy, neoadjuvant therapy, and introduction of mono-clonal antibodies and enzyme inhibitors.

Keywords: Cancer - breast cancer - leptin - BRCA1 - BRCA2 - antibodies - enzyme inhibitors

Asian Pac J Cancer Prev, **15 (8)**, 3353-3358

Introduction

Cancer is mainly caused by the mutation in genes which are present in nucleus of all cells in body. Cancer may be benign or malignant. If cancerous cells remains localized to specific organ of body, it termed as benign tumor however when these tumor cells start migrating towards other organs then it becomes malignant (Wilson et al., 2006).

Uncontrolled proliferation of cells which starts in breast cells and attains malignancy is called breast cancer. Survey has shown that breast cancer contributes 11 percent among all types of cancer diagnosed globally annually and it is a major cause of death in women. Breast cancer mainly originates from the milking ducts or the lobules responsible for milk supply towards ducts. Depending upon the origination breast cancers may be ductal or lobular carcinomas (Friedman, 2005).

Major Causes of Breast Cancer

Breast cancer is mainly caused by inherited mutations in genes which include BRCA1 and BRCA2. Family history is mainly involved in pathophysiology of breast cancer. The main cause of breast cancer is related with a personal or family history of the disease and inherited genetic mutations in the breast cancer susceptibility genes BRCA1 and BRCA2. The mutations in gene expression contribute approximately 5-10% among all cases of breast cancers (Natalia et al., 2013). Other known factors involved in breast cancer may include obesity, use of hormone therapies (progestin and estrogen), increased breast tissue density, alcohol use and physical inactivity (Emens and Jaffee, 2005).

Normally cells undergo apoptosis after completion of their life cycle when they are not further required for body. Before apoptosis they are protected by different pathways and proteins. These pathways include PI3K/AKT pathway and RAS/MEK/ERK pathway. Sometimes genes associated with these pathways become mutated and these mutations cause permanent opening of these pathways which leads to continuous cell division and proliferation and prevents cell suicide after completion of their life span. Normally PTEN protein is responsible for turning off the PI3K/AKT pathway at the time of cell apoptosis. In some cases mutations occur in PTEN protein which leads to uncontrolled proliferation of tumor cells. In breast adipose tissues over expression of leptin is also responsible for breast cancer. However, breast cancer directly linked with levels of estrogen in body (Natalia et al., 2013).

Treatment Strategies for Breast Cancer

Normally surgery, radiation therapy and chemotherapy are used to treat breast cancer. Radiations cause damage to DNA strand inside the cancer cells, which inhibits its further growth. Radiations can also damage the healthy tissues, but the effect is more on cancerous cells, as the growth of cancerous cells is very rapid and they can

*Department of Physiology and Pharmacology, Veterinary Science, University of Agriculture, Faisalabad, Pakistan *For correspondence: bilal.aslam@uaf.edu.pk*

not repair any damage easily. Chemotherapy refers to the treatment of cancer with the help of drugs which can be administered orally as well as intravenously. Chemotherapy is associated with severe side effects and is not suitable therapy for all breast cancer patients (Rita, 2012).

New Hope in Treatment of Breast Cancer

Recent research studies have introduced a new hope in treatment of breast cancer which includes adjuvant therapy, neoadjuvant therapy, monoclonal antibodies and enzyme inhibitors (Al-Mubarak et al., 2013).

Hormonal Therapy

Hormones function as chemical messengers and they show their effects at different organs in body via reaching target organs through blood stream. In females, ovaries produce two major hormones estrogen and progesterone and these two hormones are responsible for development of female sex characteristics and maintenance of menstrual cycle (Rita, 2012). However enhanced levels of these hormones can also b a leading cause of hormone-sensitive breast cancer. To identify the hormone-sensitive breast cancer surgically cancerous tissues are removed and these samples are checked for the presence of hormone receptors. If hormone receptors are present it confirms the hormone-sensitive breast cancer. Hormonal therapy reduces the development of hormone-sensitive cancerous cells by inhibiting the production of estrogen and progesterone from ovaries in body and also by inhibiting the hormonal actions (Dunnwald et al., 2007).

Ovaries are the main source of estrogen and in case of premenopausal women, high estrogen levels can cause breast cancer. It can be reduced by ovarian ablation via radiation therapy or surgery. Radiations and surgery causes permanent blockage of ovarian function. However ovarian function can be suppressed temporarily by treatment with drugs e.g. GnRH agonists or LH-RH agonists. Two FDA approved drugs are Goserelin and Leuprolide both interfere with signals from the pituitary gland that stimulate the ovaries to produce estrogen (Dunnwald et al., 2007).

Selective estrogen receptor modulators are also effective in treating the breast tumors. They show their effects by binding to estrogen receptors and prevent the actions of estrogen by functioning as antagonist of estrogen. However at the same time they also act as estrogen agonists in some other tissues. Tamoxifen is a substance which functions as selective estrogen receptor modulator. It works as antagonist in breast tissues to inhibit the tumor cells, hence also possesses agonistic potential in some other tissues e.g. in uterus and bone tissues (Vogel et al., 2006).

Aromatase is an enzyme which facilitates the formation of estrogen by ovaries and other tissues in body. In ER-positive breast cancer aromatase inhibitors are used which blocks the formation of estrogen from ovaries via blockage of aromatase enzymes. Aromatase inhibitors are more effective in postmenopausal women because the ovaries in premenopausal women produce large quantities of estrogen and blockage by aromatase inhibitors is not sufficient to block the production of estrogen. In case of premenopausal women aromatase inhibitors can be used in combination with other therapies which blocks the function of ovaries. FDA approved aromatase inhibitors are anastrozole and letrozole but they block aromatase enzymes temporarily however exemestane is also an FDA approved aromatase enzyme inhibitor which causes permanent suppression of aromatase enzymes in ovaries (Davies et al., 2011).

A meta analysis was carried out to determine the anti tumor activity of fulvestrant which is an endocrine agent and shows its anti tumor activity via destruction of estrogen. Eight studies were included in analysis. Fulvestrant was given along with other endocrine therapies for the treatment of breast cancer. Results of the study demonstrated that time to progression (TTP) of fulvestrant was similar as with other endocrine therapies. It was also observed from results that fulvestrant reduced the adverse effects associated with other endocrine therapies (aromatase inhibitors) in patients who received combined endocrine therapy (Al-Mubarak et al., 2013).

A study was carried out to compare the anticancer efficacy of fulvestrant 250mg and anastrozole 1mg. The study was conducted in postmenopausal females who experienced advanced type of breast cancer. Purpose of study was to compare the efficacy of both fulvestrant and anastrozole due to the emergence of resistance against endocrine anticancer agents. Randomized control trials (RCT) were used to compare the efficacy of fulvestrant and anastrozole in postmenopausal females with advanced breast. Results of meta analysis demonstrated that fulvestrant enhanced the duration of response in comparison with anastrozole. However in case of complete response, time to treatment failure and partial response, no significant differences were observed in both endocrine agents. From results it was concluded that both fulvestrant and anastrozole are therapeutically equivalent and fulvestrant can be used as an alternative to anastrazole in advanced type of breast cancer in postmenopausal women (Gong et al., 2014).

Adjuvant Therapy

Adjuvant therapy is the therapy which is given after main treatment (surgery) to enhance the prevention. It may include radiation therapy, chemotherapy and hormonal therapy. In case of premenopausal and postmenopausal females tamoxifen is used as adjuvant therapy which has approved by FDA as adjuvant therapy in early stage breast cancer. However FDA has also approved two other drugs anastrozole and letrozole as adjuvant therapy in treatment of breast cancer but they can only used in postmenopausal females. Another aromatase inhibitor exemestane has approved as adjuvant therapy in females who already have treated with tamoxifen (Burstein and Griggs, 2010; Regan et al., 2011).

Hormonal Therapy for Metastatic Breast Cancer

When cancer spreads from breast tissues to other tissues of body, it refers as metastatic breast cancer. Different research studies have shown that Toremifene and tamoxifen are effective in treatment of metastatic breast cancer. A study was carried out to compare the effectiveness of tamoxifen and ovarian ablation in metastatic breast cancer treatment (Howell et al., 2005). Results of study showed that seven additional patients were cured and stable treated with tamoxifen. However five additional patients were stable with ovarian ablation. From results it was obvious that prevention rate for tamoxifen was 60% and for ovarian ablation was 42% in metastatic breast cancer women. A study also showed effectiveness of fulvestrant in metastatic breast cancer in case of postmenopausal women. Another study demonstrated that aromatase inhibitors anastrozole and letrozole can be used to treat early stage metastatic breast cancer in postmenopausal women. However anastrozole and letrozole along with another aromatase inhibitor exemestane can be used in advanced stages of metastatic breast cancer (Mouridsen et al., 2003; Howell et al., 2005; Cuzick et al., 2010).

Neoadjuvant Therapy for Breast Cancer

Therapy which is given before surgery or any other main therapy is called neoadjuvant therapy. The main purpose of neoadjuvant therapy it to reduce the size of breast tumors which facilitates the surgery. Neoadjuvant therapy enhances the surgical outcomes in postmenopausal women with breast cancer. However research has shown that neoadjuvant therapy is also useful in young females with breast cancer. In earlier research studies tamoxifen was suggested as neoadjuvant therapy in postmenopausal women. However randomized clinical trials have demonstrated that aromatase inhibitors are effective as neoadjuvant therapy as they are efficient in reducing the size of breast cancer in post menopausal women with breast cancer (Chia et al., 2010).

Monoclonal Antibodies

Almost 30% of metastatic breast cancers show enhanced expression of the human epidermal growth factor receptor-2 (HER2). FDA have approved two drug therapies to suppress the over expression of HER-2 tyrosine kinase. Trastuzumab (monoclonal antibody) is used to target extracellular portion of HER-2 and lapatinib which is a direct HER-2 inhibitor. However the response with these therapies is reduced due to emergence of resistance. Resistance may be inherent or may also be produced after initial treatment with these drug therapies (Cortes et al., 2014). Another mechanism of resistance is alteration in signaling pathways and gene expressions. Resistance can be suppressed by inhibition of P-13 kinase pathways and by blockage of neoangiogenesis mechanisms. However HER-2 dimerization site targeted monoclonal antibodies and conjugate therapies can also reduce the resistance (Hurvitz et al., 2013).

In hormone receptor positive breast cancer a pathway known as P-13 kinase is very active. P-13 kinase pathway can be blocked by afinitor (everolimus) drug which is previously approved for kidney carcinomas. Afinitor gives synergistic effects when administered orally in combination with Aromasin (Exemestane) for the treatment of breast cancer. Afinitor also gives better effects when given in combination with tamoxifen. These combinations have reduced the chances of resistant for receptor positive breast cancers in females. T-DM1 is an experimental drug known as "super Herceptin. It is an antibody-drug conjugate cancer-killing agent combined with targeted antibody trastuzumab (Herceptin). A study has demonstrated that in 25% of breast cancer females who were victims' of HER2 overexpression, T-DM1 frequently overcomed the breast tumors and allowed the patients to go into remission (Rody et al., 2009; Yu-Ting et al., 2011).

In 15-20% cases of breast cancer there is over expression of HER-2 which has reduced the prognosis of breast cancer. New HER-2 targeted drug therapies have been evaluated due to the emergence of resistance against trastuzumab. A study has demonstrated the efficacy of Pertuzumab against HER-2 over expression breast cancer.

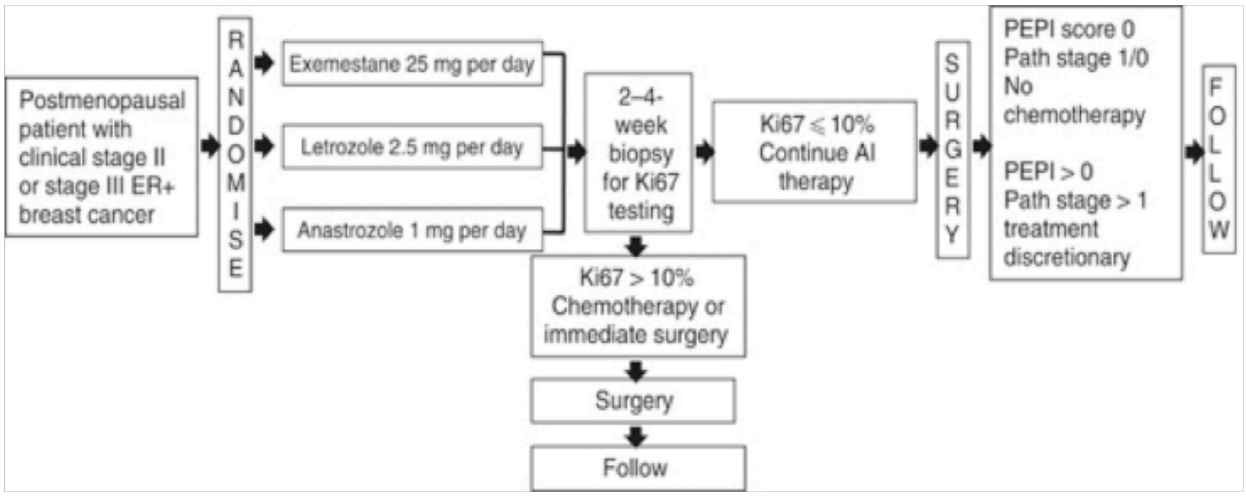

Figure 1. Diagram Demonstrates the Scheme for Letrozole and Anastrozole Trial as Neoadjuvant Therapy in Postmenopausual Females with Estrogen Receptor Positive Breast Cancer

Results of study showed that Pertuzumab has significant antitumor activity alone and also in combination with other monoclonal antibodies (Capelan et al., 2012).

Breast cancer can metastasize towards bones which causes severe pain, bone fracture and nerve compressions which may be life threatening. The mechanism involved in breast cancer metastasis towards bones involves multiple steps and results in the disruption of bone turnover. Biphosphonates have been used from last few years for the treatment of breast cancer related bones metastsis. It has been observed that biphosphonate can delay the first skeletal event and can decrease the pain. However biphosphonates do not have any preventive effect on breast cancer related bone metastasis and can't enhance the survival rate. Denosumab which is a radionuclide is a newer treatment which can also reduce the rate of skeletal morbidity along with delay of first skeletal event and also reduces bone pain (Erdogan and Cicin, 2014).

Immunotherapy in Treatment of Breast Cancer

Previously immunotherapies were not used for the treatment of breast cancer. However clinical data obtained from different studies have shown that immunotherapies have potential to improve the breast cancer related clinical outcomes and breast cancer can be considered a suitable target for immunotherapies (Ascierto et al., 2012; Soliman, 2013). In recent clinical trials immunotherapies which have shown promising results in treatment of breast cancer include.

A vaccine Nelipepimut-S is under exploration to reduce the chances of breast cancer recurrence among patients with short to transitional levels of HER2 expression following surgery. A phase III trial has carried out in 2012 to evaluate the activity of nelipepimut-S against breast cancer (Brittany and Rossman, 2013).

In a phase 2 clinical trial relating 600 females who were already treated with primary therapy and were without any kind of disease evidence, a vaccine was explored to target the AE37 peptide. In a meeting of American Society of Clinical Oncology (ASCO), which was conducted at the end of 2012, results of the trial showed that the treatment reduced the chances of cancer recurrence, with enhanced benefits in females with low levels of HER2 expression. GVAX is a type of vaccine which is made from linings of breast cancer cells, genetically engineered to secrete the immune molecule GM-CSF. GVAX has tested in a phase II clinical trial in women with stage IV breast cancer without any overexpression of HER-2. The wide antigen expression and the discharge of GM-CSF has made GVAX a perfect part of combination immunotherapy in treatment of breast cancer (Bot et al., 2013; Dassie and Giangraude, 2013).

COX-2 Inhibitors in Treatment of Breast Cancer

A randomized double blind study was carried out to determine the anti tumor activity of Cyclooxygenase-2 (COX-2) inhibitors. Thirty seven patients with breast cancer were used in study and celecoxib COX-2 inhibitor was used at dose rate of 400mg twice daily. Experiment was carried out for a period of two to three weeks. Quantitative PCR analysis was used to analyze the gene expressions. Results of the study revealed that celecoxib showed anti tumor activity by significant reduction in Ki-67 positive cells via transcriptional changes in gene expressions in primary breast cancers (Brandao et al., 2013).

Nanomedicines in Treatment of Breast Cancer

Nanomedicines can effectively utilized for avoiding all the problems associated with conventional chemotherapy. A study was carried out to evaluate the estrogen receptor targeted pH-sensitive liposomal preparation for the efficient site specific delivery of doxorubicin in the treatment of breast cancer. For intracellular delivery of doxorubicin a liposomal preperation was made by using estrone (biological ligand). Estrone was attached on the surface of liposomal preparation for intracellular delivery of doxorubicin to specific estrogen receptors. Estrone anchored liposomal preparation showed significant intracellular invading activity at acidic pH. Results of the study showed that pH-sensitive liposomal preparation showed more activity against breast cancer than non-pH-sensitive and free doxorubicin formulations. However In-vitro cytotoxic studies revealed that pH-sensitive liposomal targeted formulation is more cytotoxic than free doxorubicin and non pH-sensitive preparations via the formation of reactive oxygen species (Paliwal et al., 2012).

Enzyme Inhibitors

Radiations and chemotherapy are most commonly used therapies in the treatment of breast cancer. But now a days resistance has been emerged against these therapies in the treatment of breast cancer. This resistance is emerged due to metabolic changes in cancer cells. A study has demonstrated that almost 40% of all and 50% of advanced breast cancer cells are metabolically hypoxic which causes altered metabolism. Due to hypoxic cancer cells resistance has emerged against conventional therapies used for the treatment of breast cancer. Due to this altered microenvironment of cancer cells focus is now diverted towards the use of other therapies for the treatment of breast cancer which include different enzymes such as carbonic anhydrase IX (CAIX) (Ward et al., 2013).

MiR-886-5p Inhibitors

Mechanisms of miR-886-5p involved in inhibition of growth and migration of MCF-7 cells were evaluated in advanced breast cancer women. In study miR-886-5p inhibitors and accelerators were used to increase or decrease the miR-886-5p expressions. Rate of apoptosis, expressions of caspases 3, 8, 9, VEGF-C and MCF-7 cells secreted MMP2 and MMP9 levels (ELISA) were

measured to determine the roles of miR-886-5p inhibitors and accelerators in breast cancer treatment. Results of study demonstrated that there was a significant inhibition of MCF-7 cells growth as levels of miR-886-5p were reduced. It was also observed that reduced levels of miR-886-5p also enhanced the rate of apoptosis and caused a significant reduction in migration of MCF-7 cells. The levels of VEGF MMP2 and MMP9 were also reduced by reducing the expression of miR-886-5p. From results it was concluded that miR-886-5p inhibitors can be used as therapeutic agents in treatment of breast cancer as they caused a significant reduction in growth and migration of MCF-7 cells (Zhang et al., 2014).

Natural Products in Treatment of Breast Cancer

In connection with severe side effects of synthetic anticancer drugs, now different drugs have been derived recently from plant sources for the treatment of cancer. However in treatment of breast cancer various drugs of plant origin are in clinical development depending upon their target sites. A study was carried out to evaluate the anticancer activity of D-pinitol. D-pinitol is a drug of plant origin and it possesses different pharmacological activities. Apoptotic activity of D-pinitol was evaluated in MCF-7 cells. Different dose levels of D-pinitol were used in breast cancer patients and results were evaluated by using MTT and LDH assays. Results of study demonstrated that D-pinitol significantly reduced the MCF-7 cell proliferation in a dose dependent manner. Results also showed that D-pinitol enhanced the expression of p53 and Bax. Hence it significantly reduced the Bcl-2 and NF-kB expression (Rengarajan et al., 2014).

Resistance against anticancer drugs has become a main problem in treatment of cancer. Due to rapid emergence of resistance for chemotherapeutic agents focus is now diverted towards natural products for their anticancer potential. A study was carried out to demonstrate the anticancer potential of mangiferin in treatment of breast cancer under the assumption that it may have the ability to re-sensitize MCF-7 cells *in vitro* in those breast cancer cells which were already treated with doxorubicin. Mechanism involved in the anticancer activity of mangiferin was may be the modulation of P-glycoprotein, MRP1 and BCRP. In study the breast cancer cells which were already treated with doxorubicin were exposed to mangiferin for a period of ten days using different concentrations of mangiferin 10, 25 and 50μM respectively. In order to evaluate the anticancer potential of mangiferin viability of breast cancer cells and expressions of P-glycoprotien, MRP1, BCRP were measured. Results of the study demonstrated that at highest concentrations mangiferin it caused a significant reduction in cell viability in combination with doxorubicin. However at low doses it was failed to cause any significant reduction in cell viability. Results also revealed that at high concentrations mangiferin reduced the P-glycoprotien expression. However it did not showed any significant effects on MRP1, BCRP. It was concluded that mechanism involved in anticancer activity of mangiferin was may be its inhibitory effect on P-glycoprotiens (Louisa et al., 2014).

Studies have shown that isoflavones present in soy possesses anticancer potential. It has been observed that enhanced consumption of soy can reduce the chances of recurrence and mortality associated with breast cancer and it binds mainly with ERb receptors. Soy has both estrogenic and antiestrogenic activity (Tina and Fabno, 2012).

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