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MOLECULAR BIOMARKERS IN BREAST CANCER AS TARGETS FOR THE CHEMOPREVENTION

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ABSTRACT

Worldwide, breast cancer (comprising 22.9% of invasive cancer and 16% of all female cancer) is one of the most common invasive cancers in women. Researches ranging from identification of genes predisposing to cancer, tumor and its local environment investigation, development of cellular models, risk-factor identification, prevention models, have led to the improvement of outlook and quality of life of women with Breast cancer. The complexity of Breast Cancer presents substantial challenges for the development of new therapeutic approaches for effective prevention and control of Breast Cancer. This article aims to provide a review on few of the major biomarkers which can be used for targeting Breast Cancer based on recently published preclinical and clinical studies, and attempts to relate the basic mechanism involved with the strategy for drug design. Some of the Biomarkers which can be targeted for effective chemoprevention include ErBb2 gene, SDFα, FGFR and Cadherin, TGF-β and HER2 which are discussed separately in this review.

Introduction

Though the incidence of breast cancer and the mortality associated with the disease has decreased recently, breast cancer continues to be the most common cancer in women worldwide. Researches that range from identification of genes that predispose to breast cancer, investigation of tumor and its local environment, cellular models for pre-neoplastic diseases, identification of risk factors, possible models for prevention have been performed. Now days have improved the overall outlook and quality of life for women with breast cancer but there is a need of newer approaches for the chemoprevention of Breast Cancer [1, 2].

Some of the risk factors that predispose to breast cancer are: First-Family history-inherited genetic mutations (*BRCA-1* and *BRCA-2)* are estimated to be involved in 60%-70% of hereditary Breast Cancer [3]. Second: Ovarian hormones-prolonged estrogen exposure such as early menarche, late menopause and late age at first pregnancy, prolonged estrogen use and also use of oral contraceptives [4]. Third: If the patients have previous history of breast biopsy and lobular carcinoma *in situ* [5]. There are several genes such as ErbB2/HER2, ErbB1, ErbB3, etc whose role in breast cancer has been given, hence these genes are known as oncogenes [6, 7]. The clinical significance of ErbB2 expression has been demonstrated in ovarian carcinoma patients [8], in fact the heregulin (HRG)-induced cell proliferation in breast cancer is attenuated by ErbB2 [9]. Likewise there are numerous biomarkers which can be used for the chemoprevention of breast cancer which includes epigenetic markers, microsatellite instability, telomerase, chromosomal instability, proliferation markers, Ki-67, p53, Her-2/neu, COX-2, IGF, Cyclin D, E, Apoptotic markers, bcl-2, bax, angiogenetic markers and loss of heterozygosity [10].

Molecular mechanism of Breast Cancer (ErbB2 gene concept)

The gene ErbB2 codes for a 185-kDa transmembrane glycoprotein ($p185^{ErbB2}$), it is a protooncogene encoding a receptor tyrosine kinase which belongs to the family of epidermal growth factor receptor, located in the chromosome region 17q12 [10, 11, 12]. The oncogenecity of ErbB2 is due to various mechanisms which involves multifaceted network, the role of ErbB2 is of a ligand-less signaling subunit. The major cohorts of this gene are ErbB1 (also known as epidermal growth factor receptor) and ErbB3; it is a kinase-defective receptor and whose activation is in the milieu of heterodimeric complexes. The heterodimers escape from the normal process of inactivation by following processes: they decrease the ligand-dissociation rate and return to the cell surface to avoid the degradative pathway; they strongly conscript pathways such as the phosphatidylinositol 3-kinase mitogen-activated protein kinases and the signaling from the signaling network of ErbB and leads to the impairment of homeostasis of the cell cycle [8, 9, 13]. ErbB2/HER2 play a significant role in the development of mammary glands and alterations in the functioning of ErbB2 are commonly identified with human breast cancers [14] and if the heterodimer complex is not formed by the ErbB2 with the rest of the HER family, then the activity of HRG is blocked and cell growth is decreased which can be used as a target in the breast cancer chemotherapy [9].

Targeting the ErbB receptor for the control of breast cancer

The abnormal activation of ErbB receptors tyrosine kinase (RTK) has been consistently reported in relation to the breast cancer. The ErbB receptors are activated through both the mechanisms i.e. ligand dependent and ligand independent. After being activated these receptors stimulate signaling through tyrosine kinase activity, in turn these pathways ultimately leads to certain intracellular modifications such as proliferation and maturation. The over expression of the gene ErbB2 can be blocked by targeting this gene with trastuzumab (herceptin), a humanized anti-ErbB2 monoclonal antibody at the HER2 receptor and this technique has led to an increase in the successful treatment and survival of women with breast cancer [15-18].

There are certain other drugs which are undergoing clinical trials for e.g. various quinazoline derivatives such as ZD1839 (Iressa) which is a selective ErbB1 inhibitor and blocks the signal transduction pathways which are involved in the proliferation of cancer cell thus paving a way towards a target specific chemoprevention of the world's most common cancer [19].

Role of SDF 1α in breast cancer

Stromal cell derived factor 1 α is a chemotactic factor for T lymphocytes and was first cloned from a bone marrow-derived cell line ST2 by Tashiro *et al* [20], it acts as a ligand for the G-protein coupled receptor CXCR4, which is a 7-transmembrane G-protein coupled receptor [21-28], it was first known as a major coreceptor which was responsible for the entry of HIV [29]. It promotes the metastasis by favoring the proliferation and migration of cancer cells and CXCR4 is upregulated by hypoxia. Upon activation CXCR4 increases the mobilization of intracellular calcium and induces phosphorylation of focal adhesion kinase (FAK) and Pyk2 [30, 31]. The CXCR4 performs its signal transduction by the PI3K/AKT and MAPK pathways, which contribute to the cell migration and secretion of matrix metalloproteinases (MMP's) amongst these metalloproteinases MMP2 and MMP9 are responsible for the migration of cells through the basement membrane.[32-34].

Targeting SDF 1α for the chemoprevention of breast cancer

Since it has been proved that SDF 1α plays a major role in the metastasis of breast cancer [35-38] and the CXCR4 receptor antagonist impairs the metastasis development [39], hence this biomarker can prove to be a target which can be used for the chemoprevention of the breast cancer. It has been experimentally proved that CXCR4 receptor antagonist can be used for the prevention of the migration of metastatic cells and are also effective in fighting against breast cancer without causing any possible toxicity [29, 40, 41]. The CXCR4 receptor antagonists inhibit the SDF1α mediated chemotaxis thus preventing the invasion of the malignant cells [42]. A CXCR4 antagonist d-Arg3FC131 has been reported to bind competitively with the CXCR4 and induce apoptosis and arrest the cell cycle resulting in the inhibition of the growth and proliferation of the malignant cells [43]. It has been reported that AMD3100 a selective inhibitor of CXCR4 has been successfully used to block the activity of the CXCR4, thus preventing the migration of the malignant cells [44]. There are certain other drugs such as plerixafor, BKT140, AMD3100, AMD3465, AMD070, CTCE-9908, RCP168 MSX-122, T22, T140, TN14003, FC131, which inhibit the CXCR4 receptor and are under clinical trials [45, 46].

Vesnarinone has been reported to down regulate the activity of CXCR4 receptor and can be used effectively with conventional chemotherapy or radiation therapy [47]. CXCR4 has also been concerned with the progression of pancreatic cancer, small cell lung cancer, glioblastoma, and bladder cancer [48-51].

FGFR and breast cancer

Fibroblast growth factor receptor (FGFR) belongs to a family of structurally related tyrosine kinase receptors which is responsible for cell growth, differentiation and migration [52-56]. These are the glycoproteins which are composed of three domains *i.e.* a hydrophobic transmembrane region, extracellular immunoglobulin like domains and a cytoplasmic part which contains the tyrosine kinase domain. The FGFR family has five members *i.e.* FGFR-1 to FGFR-5 [57]. It has been demonstrated that FGFR signaling plays an important role in the survival and formation of the breast cancer cells [58, 59]. The amplification of FGFR has been linked to the effects of N-cadherin (for review see [60]). It has been demonstrated that N-cadherin stabilizes FGFR-1, so the breast cancer cells are sensitized to stimulate FGFR-2, which in turn activates the mitogen activated protein kinase (MAPK) pathway resulting in the increased expression of matrix-metalloproteinase (MMP)-9 [61, 62]. These MMP's have been previously discussed to have a role in the migration of cells through

the basement membrane [41-43].

FGFR as target for the chemoprevention for breast cancer

It has been established that FGFR can be associated with breast cancer [63-65] owing to the single nucleotide polymorphism (SNPs) and many other mechanisms which ultimately result in the cell proliferation and migration [66-71]. The major SNPs that are associated with and increase the risk of breast cancer are rs35054928, rs2981578, rs2912778, rs2912781, rs35393331, rs10736303, rs7895676, and rs33971856 within the intron 2 of the gene FGFR2 [72-75]. To target FGFR there are a lot of FGFR kinase inhibitor which are undergoing clinical trials such as dovitinib [76], BIBF 1120 [77], endostatin, and brivanib alaninate [78-80]. The failure of tamoxifen therapy has also been linked to the overexpression of FGFR4 [81], thus it can also be used as a target for the development of therapy for the patients with recurrent breast cancer. SU6668, (Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid has been demonstrated to inhibit the tyrosine kinase activity of FGFR [82].

TGF-β in breast cancer as a biomarker

Understanding breast cancer at the molecular level becomes tedious as you cannot reach to a conclusion just by estimating a single biomarker, as TGF-β one such another surrogate biomarker in the case of breast cancer. The transforming growth factors are a family of ligands that inhibits the growth and induce apoptosis in the epithelium of human colon [83, 84]. TGF – β plays a key role in the embryogenesis, response to injury, etc. It controls the homeostasis of the tissues by inhibiting the cell cycle progression, by inducing differentiation and apoptosis [85, 86]. It plays a role of tumor suppressor in the early stages of the breast cancer or metastasis but also functions as a pro-oncogenic factor in the late stages of the metastatic diseases [87, 88]. TGF – β is a group of multifunctional proteins, there are three isoforms of TGF- β i.e. TGF-β1, β2 and β3 [89, 90]. The high levels of TGF – β is found in the extra cellular matrix, they are present in their latent form which are activated by the proteases such as plasmin and these proteases are expressed by the tumor cells [91]. Although there have been many studies indicating the relation of TGF – β with breast cancer, the results of such studies have been inconsistent, few studies support the role of TGF – β in breast cancer by targeting it with some TGF – β antagonists [92-95], while some of them support that TGF – β has significant role in the early stage of the breast cancer and in the late stage of the disease this association is not statistically significant [96], hence the role of TGF – β is still inconclusive.

Conclusion

The complexity of pathophysiology of breast cancer presents substantial challenges for development of new therapeutic agents. Researches ranging from identification of genes having role in cancer, identification of risk factors, investigation of local environment, development of cellular models to the study of possible models for prevention have led to improvement in the outlook and quality of life for the patients with breast cancer. This review focuses on study of genes which may have possible role in the pathophysiology of breast cancer and can be used as target for the chemoprevention of the same & highlights the current understanding of its pathophysiology. The agents currently in use are mainly directed at symptomatic improvement and have limited efficacy both with respect to fraction of responsive patients and period over which they are effective. Thus, there is an urgent need for development of new therapeutic approaches, and this has been the focus of intensive efforts in both industry and academic sectors. The chemotherapy directed towards targeting the molecular biomarkers has proved its potential in treating various types of cancers. Thus, this review paves a way towards developing such approaches that might be more efficacious in comparison to the radiation therapy, surgery, hormone therapy, chemotherapy, immune therapy etc.

This review comes at a critical time, as the outcome of both preclinical & clinical studies doesn't provide clear insight into the

mechanism that affects breast cancer development. Real time translocation in receptors contributing potentially to the factors relating to breast cancer can serve as powerful tool for such studies. Finally with this review, we would like to stimulate a further comprehensive research into the mechanism that can potentially alter migration of malignant cells resulting in breast cancer.

Conflict of interests

Authors declare that they have no conflict of interests.

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