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Review Article

Breast Cancer Regulation and Progression: A Review on Genetics and Microenvironment of Breast Cancer

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ABSTRACT

Breast cancer is the most common cancer diagnosed among women. The stromal compartment of the breast includes the extracellular matrix (ECM) and stromal cells.

Breast cancer is complicated by unique molecular alterations and interactions of microenvironment cells and extracellular matrix. The aim of this article is to use the literature review on (BC) risk factors, pathological and molecular subtypes. Additionally, to review the progression of (BC) at molecular signatures to address the correlation between the genotypic changes and surrounding breast stroma.

The literatures covered in this review have shown that the microenvironment genetic alterations play important role on (BC) progression.

Keywords: Breast Cancer, Microenvironment, Extracellular Matrix.

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INTRODUCTION

Breast cancer (BC) is the most common cancer diagnosed among women worldwide. In 2012, around 1.7 million women were diagnosed with (BC) with high incidence seen in the developed countries and markedly lower incidence in developing countries (www.wcrf.org/). Globally, the mortality rate of (BC) is almost 521,000 deaths in 2012 with variations across the regions in the world due to better survival (WHO). Breast cancer is complicated by unique molecular signatures and diverse genetic alterations, each with distinct clinical outcomes. The microenvironment of the tumour includes cells and extracellular matrix (ECM).[1] The interaction between tumour cells and the surrounding microenvironment is important for proliferation, survival, differentiation and migration. Alterations in the tumour microenvironment have been shown to play a crucial role in (BC) progression.[1] In this review, BC risk factors, genetic abnormalities in (BC) will be reviewed. Subsequently, the development of (BC), pathogenesis and the role of the microenvironment in (BC) progression will be discussed.

RISK FACTORS

The aetiology of (BC) development is very complex caused by interaction between genetic and environmental factors. Identified risk factors for (BC) include age, reproductive factors including early menarche, late menopause, absence or short duration of breast feeding, exogenous hormones (e.g. hormone replacement therapy (HRT)), family history and life style.[2]

Age

Breast cancer incidence increases with age. Women aged 50 years and older account for 81 % of all (BC)s. The chance of developing (BC) doubles approximately 10 years after menopause. However, (BC) predominantly affects younger women (≤ 40 years) when compared to lung cancer.[2]

Reproductive factors and breast density

During a woman's reproductive life, oestrogen exposure may increase (BC) risk. In particular, women who start menarche at an early age and those who reach menopause at a late age are exposed to higher levels of oestrogen, and are more likely to develop (BC).[3] These biological causes are due to the proliferative response of breast epithelial cells to oestrogen via the oestrogen receptor (ER).[4] Later age at first full-term pregnancy also increases the risk of (BC) but the biological relationships are unclear.[3] Exposure to exogenous oestrogen such as HRT[5] and oral contraceptives[6] also increase the risk of (BC). Breast density with high proportion of non-fatty tissue increases the risk of (BC). Women with extremely dense breasts may have a 2-6 fold increased risk of (BC).[7,8] The density is affected by weight, menopausal status, number of children and inheritance.[9]

Family history

In developed countries up to 10% of (BC)s are associated with genetic predisposition. The mode of inheritance of (BC) is

autosomal dominant. Two commonly identified genes for familial (BC) are (BC) susceptibility gene 1 and 2 (BRCA1 and BRCA2), located on the long arm of chromosomes 17 and 13, respectively.[2]

Life style

Obesity has been shown to increase the risk of postmenopausal (BC).[10] After the menopause, the highest levels of oestrogen receptors are found in adipose tissue. Consumption of alcohol has also been associated with an increased risk of (BC).[11] This may be due to higher levels of sex hormones in the blood of alcohol consumers. An increased risk of (BC) occurrence has been suggested in night workers of >4.5 years shift work.[12]

BREAST CANCER SUSCEPTIBILITY GENES

Breast carcinogenesis involves a multi-step process and is thought to involve one or more distinct genetic mutations (hereditary and/or sporadic). Much molecular research and linkage has led to the discovery of the high penetrance (BC) susceptibility genes BRCA1 and BRCA2.[13] The genes and loci involved in heredity (BC) can be divided in to three groups depending on their risk for cancer development.[14] Germ-line mutations with high penetrance (the proportion of individuals carrying a specific variation and also express a related phenotype) include BRCA1, BRCA2, TP53, CDH1, STK11 and PTEN (relative risk of >5), intermediate penetrance genes include CHEK2, ATM, PALB2 and BRIP1 (relative risk of >1.5 to <5) and low penetrance genes include CASP8, FGFR2, MAP3K1 and LSP1 (relative risk of >1.01 to < 1.5).[14]

BRCA1 and BRCA2

Breast cancer susceptibility gene 1 (BRCA1), and 2 (BRCA2) are tumour suppressor genes located on chromosome 17q21 and 13q12-13, respectively.[15] BRCA1 is composed of 24 exons and encodes for a protein with 1,863 amino acids, whereas BRCA2 is composed of 27 exons and encodes a protein with 3,418 amino acids. Approximately 16% of the hereditary (BC) can be attributed to germline mutations of BRCA1 and BRCA2 genes.[16] BRCA1 and BRCA2 are implicated in DNA repair and they form a complex that initiates repair of double strand breaks (DSBs) and activate homologous recombination (HR). RAD51 along with BRCA1 and BRCA2 were co-localized at the site of DNA recombination and DNA damaged induced loci.[16] According to the database of (BC) Information Core (BIC), 1,639 and 1,853 different mutations, variants and polymorphisms in the BRCA1 and BRCA2 genes have been recorded. The types of mutation include deletions or small frameshift insertions, mutations in splice sites or non-sense mutations resulting in a complete or partial loss of exons or incorporation of intronic sequences.[16] BRCA1 and BRCA2 mutations are associated with increased risk of developing breast, ovarian, prostate and pancreatic cancer. In breast and ovarian cancer, truncated and non-functional BRCA proteins were found.[16]

TP53

The tumour protein 53 (TP53) gene is located on chromosome 17p13.1. TP53 is a nuclear phosphoprotein and functions as a transcription factor. TP53 was shown to regulate key physiological process such as the cell cycle, DNA repair, maintenance of genomic stability and apoptosis.[17] Because of its tumour suppressor function TP53 is also known as the “Guardian of the genome”. Germline mutations of TP53 are associated with Li-Fraumeni syndrome, which is associated with malignancies including sarcoma, (BC), brain tumours and tumour of the adrenal glands. The most common mutations in TP53 are point mutations, which interfere with DNA binding and activation of TP53 dependent genes.[18]

CDH1

The CDH1 tumour suppressor gene encodes for cadherin 1 or E-cadherin, and is located on chromosome 16q22.[19] E-cadherin is a cell adhesion glycoprotein and is crucial for cell to cell adhesion. Mutations in CDH1 are associated with hereditary diffuse gastric cancer (HDGC) (autosomal dominant) and increased risk of lobular (BC), with risk of (BC) in women affected by HDGC being approximately 50%[18] CDH1 mutations also increase the risk of salivary gland cancer, lung cancer and colorectal cancer.[14]

PTEN

Phosphatase and tensin homologue (PTEN) is a tumour suppressor gene located on chromosome 10q23.3 that encodes a phosphatidylinositol phosphate phosphatase.[19] The role of PTEN in cellular regulation is poorly understood. PTEN has the capacity to dephosphorylate both proteins and lipids,[21] and also act as a negative regulator of Akt phosphorylation.[22] Cowden’s disease is caused by mutation of PTEN (autosomal dominant) and is associated with increased susceptibility to developing (BC) (25-50%), thyroid cancer, endometrial neoplasm and benign hamartomas.[22]

STK11

The serine/theronine kinase 11 (STK11/LKB1) gene is located on chromosome 19p13.3.[23] This serine/theronine kinase has been shown to play an important role in cell polarisation, VEGF regulation, TP53 dependent apoptosis, and Wnt signal transduction.[24] Peutz-Jeghers syndrome (PJS) is caused by germline mutations of STK11. PJS increases the risk of cancer in diverse locations, which include the colon, breast, small intestine, oesophagus, stomach, breast, cervix and ovaries.[25]

BREAST CANCER MOLECULAR SUBTYPES

The first research article regarding the classification of (BC) subtypes based on gene expression prolifes was published by Perou and colleagues in 2000.[26] The group of intrinsic genes identified were obtained from cDNA microarray analysis of 38 (BC) cases. Hierarchical cluster analysis revealed four molecular phenotypes, which included luminal, HER2-like, basal and normal breast. Similar analysis of a larger cohort of (BC) patients have shown that the luminal group can be divided into at least two sub-types (luminal A and B).[26]

Luminal A

Luminal A (BC) accounts for 50-60% of (BC) and is the most common subtype, being histologically low in grade. The luminal epithelium in the mammary ducts is associated with expression of genes stimulated by the ER transcription factor and low expression of cell proliferation related genes.[26] Based on immunocytochemistry, luminal type A was associated with expression of ER, PR, Bcl-2 and cytokeratin CK8/18. The

expression of HER2 was absent and Ki67 was minimally expressed. The expression of the transcription factor GATA3 was also significantly higher in the luminal A subgroup. The luminal A subtype includes all cases of lobular carcinoma in situ and most infiltrating lobular carcinomas.[27] The luminal A subtype has excellent outcomes and significantly reduced relapse rates.[28] They are tamoxifen sensitive and aromatase inhibitors (AI) are used for treatment in postmenopausal women.[27]

Luminal B

The luminal B subtype accounts for 10-20% of (BC)s. In comparison with luminal A, luminal B subtypes are more aggressive, with higher histological grade and poorer outcomes than luminal A.[29,30] The main biological distinction between the two subtypes is expression of proliferation genes such as CCNB1, MYBL2 and MKI67, which is expressed at higher levels in luminal B type tumours compared to luminal A.[31] Luminal B tumours are also associated with increased expression of EGFR and HER2. The treatment of luminal B tumours is challenging as the mechanism related to survival, proliferation and metastasis is unclear. Luminal B tumours have poor prognosis despite treatment with tamoxifen,[32] and pathological complete response (PCR) was significantly lower to neo-adjuvant chemotherapy compared to other subtypes.[33]

HER2 positive

The HER2 positive subtype accounts for 15-20% of all (BC)s. HER2 is a transmembrane tyrosine kinase receptor, which belongs to the family of EGF receptors and is structurally analogous to EGFR. HER2 is encoded by the ERBB2/HER2 gene located on chromosome 17q21.[34] HER2 positive (BC)s were shown to be highly proliferative; 75% are histologically high grade and the majority have TP53 mutations.[27] HER2 positive (BC) is associated with poor prognosis, but the advent of monoclonal antibody therapy with trastuzumab (Herceptin®) has significantly improved outcomes in both early and advanced stages.[35,36]

Basal subtype or triple negative

The "basal" subtype accounts for 10-20% of all breast carcinomas. Basal types are common in young women of African origin with large tumour mass at the time of diagnosis. They are a higher histological grade and lymph nodes are frequently involved.[37] Basal subtype was associated with expression of genes characteristic of normal myoepithelial cells. The basal subtype was associated with expression of genes related to proliferation, inhibition of apoptosis and tumour invasion.[38] The myoepithelial cells of basal subtype express high MW cytokeratins CK5 & CK17, P-cadherin, caveolin 1 & 2, EGFR, nestin and CD44; whereas, genes characteristic of luminal epithelial types include CK8/18.[27] The ER, PR, HER2, EGFR and CK5/6 are the five markers used to identify the basal subtype with a sensitivity of 76% by immunohistochemistry and a specificity of 100% by microarray analysis.[29] The basal subtypes are typically negative for ER, PR and HER2, and hence, overlap with "triple negative" (ER-, PR-, HER2-) (BC)s.[27] Livasy et al 2006 evaluated the histologic and immunophenotypic properties of basal-like breast carcinomas with known microarray profile. The basal-like tumours were found to be grade 3 ductal or metaplastic carcinomas with geographical tumour necrosis, high mitotic count, expanding invasion margin and stromal lymphocytic response.[40] The visceral organs such as the central nervous system, lungs and lymph nodes are the main site of metastatic relapse.[41]

Other types

The "normal-like" (BC)s account for 5-10% of all breast carcinomas and are a poorly characterised group. They express genes characteristic of adipose tissue and do not respond to neo-adjuvant chemotherapy. They are negative for ER, PR, HER2, EFGR and CK5.[27] Another intrinsic subtype named, claudin low was recently identified (12-14%). It is associated with low expression of genes involved in cell adhesion such as tight junctions, caludin 3-4-7, occludin, cingulin and E-cadherin. They express genes related to immune response and epithelial-to-mesenchymal transition (EMT) and have a poor prognosis.[42,43]

DEVELOPMENT OF BREAST CANCER

Breast cancers are derived from epithelial cells lining the terminal ductal-lobular unit (TDLU). Breast cancer is non-invasive (carcinoma in situ) when the lesion is limited to the basement membrane of the TDLU and draining duct. In the invasive type, the cancer cells are disseminated outside the basement membrane of the ducts and lobules and invade into the surrounding adjacent normal tissue. Based on the histological pattern, invasive and in situ (BC)s are classified as ductal or lobular, depending on their growth pattern and cellular morphology.[44]

Premalignant changes in breast cancer

The spectrum of premalignant breast lesions includes atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH), ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS).[45] Atypical hyperplasia is characterised by increased numbers of cells and morphological abnormalities. DCIS and LCIS comprise malignant epithelial cells, but with an intact basement membrane and no evidence of invasion.[46]

Ductal carcinoma *in-situ*

In ductal carcinoma in-situ (DCIS), epithelial cell proliferation is limited to within the basement membrane of the TDLU and the draining duct.[44] There are several subtypes of DCIS based on morphology, which include papillary, micropapillary, cribriform, solid and comedo.[47] High grade DCIS is a common precursor for invasive breast carcinoma and recurrence in the affected breast is common.[48]

Lobular carcinoma *in-situ*

Lobular carcinoma in-situ (LCIS)is a rare form of breast carcinoma that considered to be a sign of increased risk of malignancy progression rather than a precursor lesion and it can be localised or bilaterally extensive. LCIS is composed of a uniform population of small, polygonal, round or cuboidal cells, with a high nuclear to cytoplasmic ratio and a thin border of clear cytoplasm. Based on the extent of proliferation and distension of the lobular unit ALH is differentiated from LCIS.[49]

Invasive ductal carcinoma

Invasive ductal carcinoma (IDC) derives from ductal epithelial cells and accounts for 75% of invasive (BC)s.[50] DCIS can progress to

IDC and there is a high risk of metastasis to distant organs such as lymph nodes. Tumour cell invasion begins with degradation of the basement membrane.[51] The malignant transformation of epithelial cells involves various epigenetic and genetic changes, as well as altered communication within the tumour microenvironment. The de-regulation of proliferation, differentiation, migration, impaired apoptosis and a pro-malignant stromal environment contributes to the progression of an invasive phenotype.[52]

Invasive lobular carcinoma

Invasive lobular carcinoma (ILC) was first described in 194[53] and accounts for 5-15% of invasive breast carcinomas.[50] The subtypes of ILC include alveolar, solid, histiocytoid, apocrine and signet ring.[54] Tumour size, axillary lymph node metastasis and absence of ER receptors are associated with poor prognosis.[55]

Special types of invasive breast cancer

Special types of invasive carcinoma are classified on the specific patterns of growth and cellular morphology. These include tubular, cribriform, medullary, mucoid, papillary and classic lobular.[56]

PATHOGENESIS OF BREAST CANCER

Breast progression from a pre-malignant lesion to an invasive cancer was previously considered as a multi-step process involving progressive changes from normal tissue to hyperplasia with or without atypia, carcinoma in situ, invasive carcinoma and metastasis.[57] Advances in immunohistochemistry and molecular genetics have shown that cells undergo complex genetic changes and there are divergent pathways towards invasive (BC).[45] The accumulation of various epigenetic and genetic changes and abnormal interaction with the stromal microenvironment predisposes to the transformation of normal breast epithelial cells to malignant (BC) cells. Alterations in the control of cell proliferation, survival, differentiation, cell migration and abnormal stromal microenvironment all contribute to an invasive phenotype.[52] A normally differentiated and stratified epithelium is separated by a well-delineated basement membrane from the stromal compartment. The normal stroma contains collagen bundles that surround resting fibroblasts, mature blood vessels encircled by an uninterrupted basement membrane and leukocytes such as monocytes and macrophages. In transformation to pre-malignant dysplasia, the differentiation of epithelial cells is perturbed, leading to hyperplastic epithelium. The basement membrane separating the epithelium and stromal compartment remains intact, along with activated fibroblasts and an increase in the number of macrophages.[51] In the case of IDC, the tumour cells invade the surrounding microenvironment and the myoepithelial cells along with the basement membrane are degraded. In the advance stages of (BC), the myoepithelial cell layer and the basement membrane are completely lost with invasion of epithelial cells, accumulation of stromal cells and angiogenesis.[58] The main sites of (BC) metastasis are the lymph nodes. The process of metastasis involves invasion of cells through the basement membrane, intravasation of the vasculature or lymphatic circulation, survival in the vasculature without adhesion, extrusion from the vasculature or lymphatic system (extravasate), capture at distant sites and develop into a new tumour in a remote microenvironment.[52] It has been proposed that (BC) cells with stem cell-like characteristics (dormancy, self-renewal and differentiation) promote initiation, development and relapse.[59] This is supported by the fact that long term (LT), short term (ST) and luminal or myoepithelial progenitor cells undergo several epigenetic and genetic alterations yielding several subtypes of tumours with different cells.[52]

ROLE OF THE MICROENVIRONMENT IN BREAST CANCER

The epithelium and stroma are the two main cellular compartments of normal breast tissue. The stromal compartment includes the extracellular matrix (ECM) and stromal cells.[58] The basement membrane separates the stroma from the epithelial/myoepithelial cell bilayer.[1] The basement membrane is a unique part of the ECM, composed of various proteoglycans and glycoproteins that separate epithelial cells from the surrounding microenvironment.[60] The stromal cells include fibroblasts, endothelial cells, myofibroblasts and numerous immune cells and adipocytes.[62] The ECM is a 3D structure that surrounds these cells and comprises numerous proteins such as collagens, fibronectin and laminin. The ECM is also abundant in mediators of neoplastic remodelling such as matrix metalloproteinases (MMPs) and soluble growth factors.[58] It has been reported extensively that the microenvironment plays a pivotal role in modulating important aspects of mammary epithelial cells such as proliferation, polarity, differentiation, survival and metastatic capacity of mammary epithelial cells.[1] The tumour microenvironment was proposed to be actively involved in (BC) pathogenesis supported by a striking difference in molecular signatures between the stromal cells of tumours and normal breast tissue.[58] Cell to cell, and cell to microenvironment interactions are implicated in this process, but the mechanisms are poorly understood.[1]

Fibroblasts

Stromal fibroblasts play a crucial role in the pathogenesis of (BC). Cancer-associated fibroblasts (CAFs) secrete ECM proteins and mitogens, which are important for tumour growth and metastasis.[61] Fibroblasts have been shown to regulate several functions in tumours such as cell to cell interaction, adhesion, migration, ECM degradation, transcriptional regulation and paracrine signaling. Expression of fibulin1 (FBLN1), kruppel-like factor 4 (KLF4), TGFβ2, Wnt1 inducible signaling pathway protein 1 (WISP1), plasminogen activator inhibitor 2 (PAI2), and tissue plasminogen activator (PLAT) is deregulated in CAFs.[62] A co-culture model has compared the luminal cells, myoepithelial cells and stromal fibroblasts between normal and malignant breast tissue. The 3D heterotypic culture system containing mixed normal or tumour cells has shown that these cells arrange into structures recapitulating normal and DCIS breast, with myoepithelial cells oriented around the luminal population. The tumour cell co-units show an altered basement membrane with loss of β4-integrin corresponding to DCIS. The addition of CAFs compromised the co-unit organisation, which was prevented by MMP and/or c-met inhibitors.[63]

Myoepithelial cells

Myoepithelial cells have been shown to express various tumour suppressor proteins, angiogenesis inhibitors, proteinase inhibitors and ECM structural proteins.[58] The normal myoepithelial layer is an endogenous tumour suppressor in both a paracrine (bFGF,

TGF-α, and IL-6) and an autocrine manner (resistance to transformation).[64] Because of their tumour suppressor function myoepithelial cells are also known as the "Cinderella" of the breast.[65]

Myofibroblasts

The stromal compartment of the tumour also includes myofibroblast, which are stimulated fibroblasts and express α-smooth muscle actin (α-SMA). The levels of VEGF, bFGF and Ki-67 were significantly up-regulated in myofibroblasts from human tissue sections of invasive (BC). The expression of the above mentioned factors in myofibrobaslt in (BC) was shown to predict shorter overall survival and relapse-free survival.[66] α-SMA-positive myofibroblasts were also shown to promote tumour invasion and angiogenesis in mouse gastric cancer.[67]

Endothelial cells

Endothelial cells are recruited to the tumour stroma where they play a critical role in angiogenesis, tumour growth and metastasis.[58] The presence of endothelial cells has been shown to promote the angiogenic activity of malignant mammary epithelial cells.[68] Apart from transporting nutrients and oxygen, breast endothelial cells are important components of the microenvironment and provide growth signals to both the normal and malignant breast epithelium.[69]

Adipocytes

Adipocytes have also been implicated in the pathogenesis of (BC) and have been shown to promote the invasive capacity of (BC) cells.[70] Co-culture of adipocytes with cancer cells increased their invasive capacity. Moreover, the modified adipocyte phenotype was characterised by low expression of adipocyte markers and lipids, with over-expression of MMPs and inflammatory markers.[70]

Bone marrow derived cells (BMDCs)

Bone marrow derived cells (BMDCs) such as mast cells, lymphocytes, macrophages and neutrophils are recruited by primary tumour cells to promote migration, angiogenesis and invasion.[58] These immune infiltrates are frequently observed at the site of degraded myoepithelial cell layers, suggestive of their role in invasion and metastasis.[71]

EXTRACELLULAR MATRIX (ECM)

The extracellular matrix (ECM) functions as a scaffold to maintain tissue and organ structure. The ECM is formed by the assembly of numerous proteins and polysaccharides forming an extensive meshwork within tissues, being primarily composed of fibrous structural proteins which include collagens, laminins, fibronectin, vitronectin and elastin. The ECM is also made up of some specialised proteins (growth factors, small matricellular proteins, small integrin binding glycoproteins) and proteoglycans. The composition of ECM components is variable depending on the type of tissue. Apart from providing mechanical support to the tissues, the ECM also regulates pivotal physiological processes such as cell proliferation, migration, and differentiation, alteration in cell shape, growth and survival.[72] The ECM undergoes constant remodelling by degradation and reassembly and is intensified during development, wound repair, infectious diseases and in many other disease states. Integrins are heterodimeric trans-membrane receptors, which play an important role in the transduction of signal from the ECM to the cell interior. The signal transduction by integrins through ECM proteins significantly influences gene expression patterns and important cellular processes such as angiogenesis, cell proliferation and invasion. The ECM can be modified in response to signals transmitted by ECM receptors such as integrins, laminin receptors, syndecans and proteases such as matrix metalloproteinases (MMPs).[72] In tumours, tissue homeostasis is deregulated due to diminished cell adhesion signalling, cytokine/growth factor independent growth and disrupted ECM signaling.[73] Integrins have been shown to play an important role in transduction and integration of signals from the microenvironment. Integrins are heterodimeric transmembrane proteins and made up of α- and β- subunits. Approximately 24 integrins with various subunit combinations have been reported. The integrins were shown to regulate various carcinogenic processes such as proliferation, differentiation, survival and migration.[74] Integrin α9β1 was implicated as a novel marker in basal (BC)s and it augments cell migration and invasion. Integrin α9β1 expression in (BC) was associated with poor prognosis.[75] It remains elusive how changes in ECM composition and signalling mechanisms contribute to tumour progression.[72]

CONCLUSION

This review has showed that the microenvironment cells interactions and molecular genetics alterations influencing (BC) progression and warrants further investigation to elucidate the pathways on which they act in tumorigenesis.

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