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Molecular genetics of breast cancer: Possible clinical implication

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INTRODUCTION

Breast cancer is one of the most common cancers diagnosed in women worldwide and a leading cause of female cancer-related death, despite improvements made in the field of risk factors definition, early detection, diagnostics and the treatment of the disease. In Yugoslavia estimated breast cancer incidence per 100 000 women (according to data of Population-based Cancer Register in Sremska Kamenica, 2000) is about 3890 cases.

As for all other malignant tumors, breast cancer carcinogenesis represents a multistep process associated with accumulated genetic aberrations - probably with 5 to 6 specific mutations per somatic cell. Considering the rarity of mutations in normal cells (mutation rate in normal tissue is very low - about 1 in million per gene per cell) and the large number of mutations observed in human cancers, it can be proposed that spontaneous mutation rate in normal cells is not sufficient to account for such large number of mutations in a single cancer cell. But, the cancer occurs because of combination of two mechanisms: firstly, some mutations enhance cell proliferation, increasing on that way target cell population for the following mutations and secondly, some mutations generate genome instability increasing the overall mutation rate. Mutations in genes that function in the maintenance of genomic stability are manifested by increases in mutation rate in cancer cells that drive tumor progression.

Breast cancer genes can be defined as the genes in which germline mutations or polymorphism confer increased susceptibility to breast cancer. With this definition only the hereditary breast cancer is covered. However, in a broader sense, breast cancer genes are genes that play a causative role in the pathogenesis of breast cancer even if they do not have any germline or somatic sequence alterations. In this group genes that are aberrantly expressed in breast tumors due to epigenetic changes (i.e. aberrant methylation) are also included.

GENES INVOLVED IN BREAST CANCER

In general, in the basis of malignant transformation lies in abrogation of

the balance between cell proliferation and cell apoptosis. Cells with genetic abnormalities survive due to defects in apoptotic pathways. Consequently, alterations of the genes regulating processes of cell proliferation and cell apoptosis are involved in the development of cancer. Three major classes of genes are described:

- Oncogenes, which are normally positive regulators of cell proliferation and have to be activated;
- Tumor suppressor genes, which are normally negative regulators of cell proliferation and have to be inactivated;
- DNA repair genes, which have to be inactivated, affecting on that way mutation rate in oncogenes and tumor suppressor genes.

Most breast cancer is due to somatic genetic alterations that are specific to breast epithelial cells, many of which are probably still unknown. Identifying and characterizing somatic genetic alterations that are rate-limiting step to carcinogenesis relies increasingly on genetic approaches, including genomic comparison of tumor and normal tissues genes at each stage of tumor development. It can be expected that with the reached technological improvement concerning microarray technique, tissue and tumor stage specific genes will be characterized. Till then, with the exception of hereditary breast cancer with unique well-defined genes BRCA1/2, common gene changes shared with other cancers are described as possible markers for early detection, prognosis of disease and therapeutic predicting of sporadic breast cancer.

ONCOGENES

Among the oncogenes intensively studied in last few years, are the members of ras, her and myc families.

Ras family is activated by point mutations. The ras gene product is monomeric membrane-localized protein with GTPase activity that functions as a molecular switch linking receptor and non-receptor tyrosine kinase activation to downstream events. Human cells contain at least three distinct ras oncogenes (K-, H-, N-) encoding closely related proteins. Ras-dependent signaling may mediate differentiation or proliferation, due to the cell type and transmembrane receptor - briefly, ras-effector pathways mediate its effects on progression through the cell cycle. Activating mutations in Ras protein result in constitutive signaling, thereby stimulating cell proliferation and inhibiting apoptosis. H-ras induces invasive breast cancer phenotype in breast epithelial cells (1). Further more, it has been shown that the ras signal transduction cascade may share common elements with integrin-signaling pathway. Cells with mutated ras oncogene also increase the expression of enzyme metalloproteinases, connected with metastatic ability, as well as the expression of angiogenic growth factor VEGF, connected with angiogenic ability.

Her oncogenes (EGFR/Her-1 and c-erbB-2/Her-2) family consist of four members - EGF-R (c-erbB-1), c-erbB-2, c-erbB-3, c-erbB-4. Despite extensive structural homology these receptors differ in their ligand specificities. EGF-R binds multiple distinct growth factors that share an EGF-like motif such as EGF and TGF α . Among c-erbB ligands heregulin is well defined. Her family is activated by gene amplification, which lead to overexpression of their protein products. Functionally, they encode growth factor receptors with tyrosine kinase activity. Her receptors can be changed to become constitutively activated and accordingly independent of ligand binding thus contributing in carcinogenesis. Amplification of c-erbB-2 gene, which usually results in overexpression of the encoded transmembrane protein p185, occurs in about one third of breast cancers (2). c-erbB-2 altered breast cancer is associated with decreased survival, thus exhibiting poor prognosis (3). Concerning its predictive value, breast cancer with amplified c-erbB-2 manifests lower responsiveness to methotrexate-based regimens and endocrine treatment with tamoxifen, as well as higher responsiveness to doxorubicin-based regimens (4,5).

Myc family is activated by gene amplification. c-myc gene participates in most aspects of cellular function, including replication, growth, metabolism, differentiation and apoptosis. It is an estrogen-responsive gene. However, data pertaining to several fundamental questions about the functions of c-myc such as its role in early and/or advanced stage of breast carcinogenesis exist.

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Several reports show an association of c-myc gene amplification with a poor prognosis of breast cancer, whereas many others do not find such correlation. On the contrary, higher c-myc mRNA levels in breast cancer are correlated with better survival (6).

TUMOR SUPPRESSOR GENES

Among tumor suppressor genes, the most prominent is p53 gene, which is mutated in about one third of breast cancer. Mutations of p53 in breast cancer appear to cluster in exons 5 through 9 and are associated with high histologic grade and clinical aggressiveness. While most p53 abnormalities occur as spontaneous somatic events, patients with germline p53 mutations (Li-Fraumeni syndrome) also have an increased incidence of breast cancer. A relationship between BRCA1 and p53 in hereditary breast cancer, where p53 acts as a cancer cofactor, in these patients has been presumed. Because of its function in regulating cell proliferation, maintenance of genome integrity, as well as in apoptosis, it is investigated as a possible prognostic and predictive factor. p53 appears to be useful prognostic marker, particularly in node-negative breast cancer patients, and may also help identify patients more likely to respond to chemotherapy or radiotherapy (7). However, p53 has a dual and complex role in chemosensitivity; it can either increase apoptosis or arrest growth and thereby increase drug resistance. This may explain why promising preclinical data indicating that presence of wild type (wt) p53 would predict chemosensitivity to agents acting via p53-dependent apoptosis translated into more inconsistent clinical data.

Minorities of breast cancer patients with striking family history, suggestive of Mendelian inheritance, demonstrate the presence of hereditary breast cancer (HBC). HBC is associated with germline mutations in BRCA1 and BRCA2 genes. Mutations are located throughout BRCA1/2 genes with little evidence for clustering or "hot spots". Because BRCA1 and BRCA 2 genes encode proteins that normally function to mediate genetic integrity after DNA damage, they are known as tumor suppressor genes. The discovery of BRCA1/2 genes that have been implicated in up to 40% of HBCs, made possible testing for genetic predisposition to develop disease. A woman with BRCA1/2 mutations has life-time risk for developing breast cancer up to 85%. For BRCA1/2 mutation carriers the disease tends to have strikingly early age of onset, with 50% of cases diagnosed by age of 41 years.

DETECTION OF MICROMETASTASES BY MOLECULAR BIOLOGY TECHNIQUES

Important use of molecular biology in early detection of breast cancer concerns characterization by molecular techniques. The advantage of this molecular approach is possibility of identification of very small numbers of tumor cells in a heterogeneous population of cells, i.e. molecular method poses higher sensitivity than the classical ones (histopathology, immunochemistry). Since the goal of adjuvant therapy in cancer treatment is eradication of micrometastases before metastatic disease becomes clinically evident, such early detection of metastatic process can identify the patients who are most or least likely to benefit from adjuvant therapy. Molecular detection procedures are used to identify residual tumor cells in bone marrow, lymph nodes and peripheral blood. Very attractive approach, proposed as readily accessible biological parameter that may be useful to monitor disease progression, is to measure gene expression on mRNA level of the genes normally non-expressed in tissues searching for metastases. The intermediate filament protein CK 19 is specific epithelial marker, which can be detected on mRNA level by RT-PCR in the blood, bone marrow and lymph nodes of the patients with epithelial tumors such as breast (8). The specificity of RNA-based markers is not absolute due to possible presence of the low level of illegitimate expression of the marker gene in the surrounding tissue.

THERAPEUTIC TARGETING

Therapeutic targeting has also derived from genetic analysis of breast cancer. Numerous clinical investigations are conducted. For example, interrupting the ras-signaling pathway has been a major focus of new drug-development efforts due to the high percentage of human tumors harboring oncogenic ras mutations. The prevention of membrane localization of Ras protein with farnesyl transferase inhibitor (FTI), signed as R115777 in patients with metastatic breast cancer is investigated. Preliminary results of a phase II study have recently been reported (9).

Very recently, new molecular therapeutic approaches for breast cancer have become possible. The most fully developed model thus far is treatment of some advanced breast cancer with an antibody to the protein product of the Her-2/neu gene expressed on the surface of some breast tumors. The Her-2/neu model exemplifies a large class of future translational research that has to:

- Identify a gene differentially expressed in breast cancer vs. normal breast epithelial cells;
- Characterize the gene and its product;
- Identify the biological role of the gene in tumorigenesis;
- Develop an antibody (or in principle another molecule) to block activity of the protein;
- Evaluate the approach in the clinical setting.

In the future, with understood biological pathways, based on a particular genetic alteration, the efforts must be directed toward identification of target molecules that have to be used as agents of prevention, detection and therapy.

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