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Breast cancer susceptibility gene 1 - BRCA 1

ABSTRACT

Breast cancer is the most common cancer in human population and second-leading cause of cancer mortality in women with approximately one in nine being affected in their lifetime. Epidemiological studies have shown that 5-10% of all breast cancer cases are thought to be hereditary. Linkage analysis have provided the evidence for the existence of several breast cancer susceptibility genes. One of them, BRCA1 (BReast CAncer susceptibility gene1) has been mapped in 1990 and detected by positional cloning in 1994. It spans approximately of 100kb of genomic DNA organized in 22 coding and 2 non-coding exons. Product of BRCA1 gene is protein of 1863 amino acids with still unknown function. The mechanisms of its biological function(s) is subject of intensive research. Germ-line mutations in the BRCA1 gene can be found in 45% of families with significantly high incidence of breast cancer and at least 80%-90% of families with an increased incidence of both breast and ovarian cancer.

Key words: *BRCA1 gene; Breast cancer; Ovarian cancer; Cancer susceptibility genes*

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INTRODUCTION

The first report of HBC (Hereditary Breast Cancer) was published by Paul Broca, the French surgeon, in the mid-1860. He has described, for the first time, a family with multiple cases of breast cancer in different generations.

Today, current epidemiological definition of HBC is: a breast carcinoma patient with a positive family history of breast carcinoma and sometimes related cancers (particularly ovarian carcinoma) with high incidence and distribution in the pedigree (more than three relatives being affected) that are consistent with an autosomal dominant highly penetrant susceptibility factor (1). This definition includes HBOC (Hereditary Breast Ovarian Cancer) syndrome as well. Around 5-10% of all breast carcinoma cases are thought to be hereditary.

On the other side, there is FBC (Familial Breast Cancer) which is very often mixed up with HBC or HBOC syndrome. FBC can be defined as a breast carcinoma of a patient with a family history of one or more first or second degree relatives who do not meet more rigorous HBC definition (1). As many as 26% of breast cancer cases may be FBC.

Study of HBC and FBC were significantly advanced after mapping the susceptibility locus on 17q21 (2). Four years after that, BRCA1 gene was identified by positional cloning (3). Soon afterwards, a complete coding sequence has been read. It has been shown that BRCA1 gene represents a large size gene, around 84 kilobases of genomic sequence. It consists of 7800 nucleotides organized in 22 coding and 2 non-coding exons. BRCA1 mRNA codes a polypeptide of 1863 amino acid residues showing no homology with any known proteins.

Although the precise biological function of BRCA1 protein is still unknown, it has been suggested that it belongs to a group of tumor suppressor genes described as genome caretakers influencing the maintenance of genome integrity (4). BRCA1 protein plays a crucial role as a suppressor of cell proliferation. Antisense BRCA1 oligonucleotides designed to bind and inhibit BRCA1 mRNA, when applied, accelerated the growth of normal and malignant epithelial cells (5). Similar result was obtained with NIH3T3 fibroblasts (6). Transfection of breast and ovarian cancer cells deficient in BRCA1 mRNA with wild type BRCA1 gene produced the restoration of normal growth control and loss of malignant potential (7).

Up today, three short regions have been detected within BRCA1 protein that share homology with specific functional domains of human proteins: Zinc-RING finger domain, 2 NLS (Nuclear Localisation Signal) and putative C terminal activation domain with two BRCT regions.

Zinc-RING finger domain is localized at the N-terminal end of BRCA1 protein (3). Although around 80 Zinc-RING finger containing proteins have been described to date, exact biological function of the domain has not yet been identified. Initially, it was suspected that the function of Zinc-RING finger domain of BRCA1 protein is specific binding to DNA (2). Later on, BRCA1 has been reported to associate in vivo to the RNA polymerase II holoenzyme complex (8). In addition to a possible function of BRCA1 protein, two new proteins, BARD1 (9) and BAP1 (10), that interact with Zinc-RING domain of BRCA1 protein have been detected using two hybrid system techniques. All those findings rise the possibility that BRCA1 might associate to specific DNA structure either alone or in formation of a complex with BARD1 or BAP1 proteins in a process of transcriptional activation.

On the C-terminus of BRCA1 protein, two BRCT regions are localized. They are presented with stretch of 70 acidic residues responsible for negative charge. Tandemly repeated BRCT motifs are homologous to a motif present in proteins responsible for cell-cycle check points. It has been shown that when fused to GAL4-DNA

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binding domain, carboxy- terminal portion of BRCA1 protein acts a strong transcriptional transactivator of the reporter gene (11).

Central region of BRCA1 protein harbors a binding site for Rad51 protein, an eukaryotic homologue of RecA protein of E.coli. Rad51 is involved in repairing double-stranded DNA breaks (12). Since BRCA1 and Rad51 co-localize in nucleus with the highest level of expression in intensively proliferating tissues, it has been suggested that BRCA1 together with Rad51 is involved in process of DNA repair.

Investigations on sub cellular localization of BRCA1 protein still give conflicting data. Normal BRCA1 protein contains at least two NLS and it has ability to translocate to the nucleus. It has been shown that BRCA1 interacts with the importin-a subunit of the nuclear transporting signal receptor (13). However one group has reported that BRCA1 protein is normaly nuclear but has aberrant localization in breast and ovarian cancer cells where it is cystoplasmic (14). Regarding those facts tigether with other characteristics of BRCA1 protein, it can be assumed that BRCA1 protein acts as a transcriptional activator. But, it has to be underlined that those conflicting results about its localization may be a result of a different nature of used antibodies (15).

Biochemical studies have revealed that BRCA1 protein expression is cell-cycle regulated and that at the same time BRCA1 protein undergoes different levels of hyperphopshorylation. BRCA1 levels are the highest during S phase progression and remain elevated in M phase. Changes in BRCA1 level are parallel with its level of hyperphosphorylation and correlate with changes in mRNA levels. One of the function of BRCA1 protein with respect to the cell-cycle depending manner of its expression and hyperphosphorylation might be in cell cycle regulation of G1-S transition and/or G2-M phase (15).

A role of BRCA1 protein in apoptotic signal pathway as well as an important role during the embriogenesis has also been suggested (16,17).

EPIDEMIOLOGY OF HEREDITARY BREAST CANCER

First empiric data recognizing clustering of multiple breast cancer cases within one family date 70 years back (18). Since then, family history of the disease has been the most documented risk factor for breast cancer appearance. Increased risk of breast cancer is associated with positive family history for ovarian cancer and both are associated with positive family history for prostate cancer in men and colon cancer. Moreover, when family history includes more the second or the third degree relatives, breast or ovarian cancer risk is lower comparing to families with numerous breast or ovarian cancers within the first degree relatives. Approximately, 5-10% of breast cancer cases are hereditary. Population based studies of families suggested that breast cancer susceptibility is due to inheritance of autosomal dominant allele. Those susceptibility alleles are rare in general population with frequency varying between 1:800 (19) to 1:300 (20). Breast cancer rarely occurs among men carrying BRCA1 susceptibility alleles, but among women who inherit one or two of these alleles, risk for developing breast or ovarian cancer is 80-90% during the life time.

BRCA1 mutations account for around 45% of families with a significantly high incidence of breast cancer and at least 80% of families with an increased incidence of both breast and ovarian cancer (21). But, proportion of high-risk families with breast and/or ovarian cancer attributable to BRCA1 mutations varies among populations being highest in Russia with 79% of high-risk families and the lowest in Belgium, Holland, Germany, Norway with less than 20% of the families (22). Preliminary studies carried out in Central-Eastern European populations (including Yugoslavia) suggest a relatively high mutation frequency among moreate-risk breast/ovarian cancer families. Those differences represent historical influences of migration, geographic or cultural isolation and population structure (22).

MOLECULAR EPIDEMIOLOGY OF BRCA1 GENE

First collaborative study of BRCA1 germline alterations has been performed in 1995, when among 372 unrelated families 63 different BRCA1 alterations were found (26). Those alterations were spread across the entire coding region of the gene as well as in noncoding regions. The majority of those alterations were nonsense, frameshift, splice or regulatory mutations giving truncating protein product. Smaller amounts of detected mutations were missense mutation and other variants with unknown effect on protein function.

To date, more than 300 different alterations have been detected and the number is still increasing (Breast Cancer Information Core-BIC). Mutation spectra studies of BRCA1 gene have revealed that the majority of mutations lead to a loss of function and include deletions and insertions leading to frameshift and nonsense mutations. Deletions and insertions constitute around 60% of all detected mutations in BRCA1 gene. Concerning the missense mutations, majority of them are represented by G:C to A:T transitions (17%) and A:T to G:C transitions (12%) (27).

Some of detected mutations have been localized in regulatory elements of BRCA1 gene and they are represented with large germ-line rearrangements (28, 29).

In contrast to other cancer susceptibility genes, somatic mutations in BRCA1 gene are very rare events in breast or ovarian cancers. Alternative mechanisms of inactivation have been proposed but currently there is no strong evidence that these genes are involved in sporadic form of the disease (30). On the other side, sporadic breast carcinomas show significant frequencies of LOH (Loss of Heterozygosity) at the 17q locus (31). This fact suggests that BRCA1 is a tumor suppressor gene. Mutation in BRCA1 gene predispose to breast cancer but they are not a direct cause of breast cancer appearance. Its mutations may be a non-obligate, non-sufficient event in multistep breast cancer pathogenesis.

Beside the fact that more than 300 germ-line mutations have been found in BRCA1 up today, a number of possible founder effects (alleles likely originated from a single, mutational event in the distant past) have been described for BRCA1. The best example is in Ashkenazi Jewish population where 185delAG appears with >1% frequency in general population while 5382insC occurs at a population frequency of 0.11% (32).Those two mutations, 185delAG and 5382insC, occur with considerably high frequencies among populations and constitute about 20% of the total mutations reported so far (27).A strong founder effect has also been reported in Central-Eastern European region. The observed high variability concerning the geographic and ethnic distribution of some ancient mutations and the proportion of breast/ovarian families attributable to BRCA1/BRCA2 as well as the ratios of the recurrent/unique mutations in Europe emphasizes the importance of studies of yet uninvestigated European populations (23).

PHENOTYPE OF BRCA1 ASSOCIATED TUMORS

There are several evidences that HBC, HBOC and FBC have different phenotypes comparing to sporadic cases. There are strong suggestions that a reason for this is associated with different mechanisms of development of those two forms.

BRCA1 associated tumors are mostly medullar or atypical medullar with early age of onset and high grade. They are highly proliferative and poorly differentiated with high mitotic grade and tumor aneuploidy (33, 34). It has also been found that BRCA1 associated tumors show estrogen receptor negativity.

BRCA1 associated breast cancer patients have no worse prognosis comparing to breast carcinoma patients at large. They can be said to be far better (34). These observations are paradoxal because aneupolidy, estrogen receptor

negativity and high mitotic grade are characteristics of very aggressive cancer phenotypes. A clue for this paradox may be hidden in the presence of medullar and atypical medullar carcinomas, which are significantly observed among BRCA1 associated carcinomas. Medullar carcinoma is a subgroup of high grade breast carcinoma that is frequently estrogen negative but with a significantly favorable prognosis comparing to ductal or lobular types. Thus, estrogen receptor status could be regarded as a feature of phenotype triggered by the BRCA1 germline mutation, much more than the result of tumor progression (35).

FUTURE PERSPECTIVES

This year marks the 10th anniversary of the mapping the breast-ovarian cancer susceptibility gene BRCA1 to chromosome 17 (2). This discovery together with the discovery of other breast/ovarian cancer susceptibility gene BRCA2 has placed inherited susceptibility for breast and ovarian cancer to the center of breast cancer research. Understanding the biological mechanisms that underline the susceptibility genes, has become a major research activity as well as mutation testing with the prospects for improved prevention and treatment of the disease in women at high risk (36).

There is still room for several predisposing genes not yet discovered (usually marked as BRCA3, BRCA4…) causing substantial relative risk. Discovery of such genes could explain the familial risk and many multiple case families not due to BRCA1 and BRCA2. A large number of such studies have already been undertaken investigating candidate loci and genes.

A strong breakthrough in the field of breast cancer research and treatment has been made by constructing an Xu mouse using conditional knockout approach to mutate the intact allele in the mouse bearing heterozygous deletion of Brca1 (37). Since mice bearing homozygous null mutation in Brca1 die in early stages if embriogenesis and mice heterozygous for Brca1 deletions do not develop mammary tumors, investigation of molecular mechanism of BRCA1 involvement in breast cancer was impossible. The Xu mouse represent the first model in which the mechanisms of genetic instability and result tumorigenesis in the Brca1-deficient mammary gland can be studied (38). This alone will make a very useful model for understanding how cells that have lost Brca1 function become transformed and for the testing treatments aimed at blocking that process (38).

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