



Breast cancer susceptibility Gene 2 - BRCA2

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Epidemiological studies show that 5-10% of all breast cancer cases are thought to be hereditary. When BRCA1 gene was cloned in 1994, a second breast cancer susceptibility locus was mapped on chromosome 13q12. BRCA2 was cloned in 1995, and named breast cancer susceptibility gene 2. It spans around 70 kilobases of genomic DNA and consists of 27 exons. The product of BRCA2 gene is protein of 3418 aminoacids. It looks like that the major function of BRCA2 protein is in DNA repair process. Germline mutations of the BRCA2 gene account for the majority of families with both female and male breast cancer. The risk of breast cancer among men is higher for BRCA2 and the risk of ovarian cancer is not so elevated comparing to BRCA1 gene.

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INTRODUCTION

The evidence for the existence of second major breast cancer susceptibility gene emerged from the analysis of 214 families linked to chromosome 17q in 1990, and after the localization of BRCA1 (1). In this study, only about 50% of the families with breast cancer showed the linkage to BRCA1 locus although almost all families with breast and ovarian cancer were due to this gene. An additional evidence supporting this hypothesis came from the study of families with at least one case of male breast cancer and several cases of female breast and ovarian cancers (2). In 1994, a second breast cancer susceptibility locus was mapped to chromosome 13q12 (3) and soon afterwards, using a positional cloning, a new gene was identified and named BRCA2 (4). BRCA2 gene is composed of 27 exons of which the first is non-coding (5). Like in case of BRCA1 gene, again exon 11 is unusually large with more than 5kb. mRNA of BRCA2 gene encodes a polypeptide of 3814 amino acids with no sequence similarity with any other known protein.

Up today, two structural features have been discovered that may provide clues for function of BRCA2 protein .

First, the region close to the C-terminus of the protein contains six out of the seven conserved amino acids characteristic for granin domain. But as a difference to other proteins containing granin motif, BRCA2 protein does not have hydrophobic signal sequence. Hence, the function of this granin motif is yet to be determined.

Second domain has been discovered inside the region of protein coded by the exon 11. It consists of eight copies of 20-30 amino acids sequence termed BRC motif. The repeats are separated by the stretches of 60-300 amino acids. This structure motif is not similar to any previously reported in protein database. The region encoded by the exon 11 is poorly conserved among species, but BRC repeats are among a few highly conserved regions (6). However, the function of BRC motifs is still unknown.

A portion of BRCA2 protein encoded by its third exon shares homology with a known transcription factor and is capable to activate transcription indicating one of the possible functions of BRCA2 protein. This region is highly conserved among human and mouse and it shows some sequence similarity to the activation domain of c-jun. Transcriptional activity assay showed that this region has potential to activate transcription in some mammalian cell lines when fused to GAL4 DNA-binding domain (7). At the same time, other highly conserved domains of BRCA2 protein did not show that activity. This finding suggests that BRCA2 protein might function as a transcription factor in the regulation of gene expression.

One of the very important features of BRCA2 protein is its direct

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association with Rad51 reported by different groups (8). hRad51 is human homologue of E.coli recombination and repair protein RecA. It catalyzes ATP-dependent DNA strand exchange reaction and it has a role in recombination and DNA repair. First suggestion about the involvement of BRCA2 protein in the response to DNA damage was made by Sharan et al. (8) who observed that embryos with disrupted BRCA2 were hypersensitive to ionizing radiation, just like Rad51-null embryos.

Besides the fact that they interact directly through at least two binding sites positioned in the N-terminal end of the region coded by exon 11 and in the C-terminal region of the BRCA2 protein, additional data support this hypothesis. Those two proteins colocalize and they have the same expression pattern during embryonic development.

Recent findings reported by Siddique et al. (9) demonstrate a strong HAT (Histone Acetyl Transferase) activity on the N terminus of BRCA2 protein. The part of BRCA2 protein encoded by exon 3 and responsible for the transactivation function of BRCA2 is not needed for HAT activity function, so this activity is probably enabled by separate functional domain. Results also demonstrated that BRCA2 protein acetylate primarily H3 and H4 fraction of free histones. HAT activity may play a crucial role in the tumor suppressor function of BRCA2 gene.

Because of the large size and obviously possible multicellular function of BRCA2 protein including DNA repair, transcriptional activation and HAT activity, it is reasonable to postulate that BRCA2/Rad51 complex regulates expression of genes involved in growth control, differentiation and apoptosis. BRCA2/Rad51 complex may use the HAT activity to destroy nucleosomal structure to recognize DNA damage for DNA repair.

The expression of BRCA2 mRNA is cell cycle regulated and associated with proliferation in normal and tumor derived breast epithelial cells. Cells arrested in G0 or early G1 phase have the lowest level of BRCA2 mRNA. During G1 phase the level of BRCA2 mRNA is rising and reaching maximum level in late G1 phase and during S phase (10). It looks like that kinetics of BRCA2 mRNA up-regulation appeared to be similar to those of BRCA1, suggesting that two genes can be commonly controlled and regulated.

MOLECULAR EPIDEMIOLOGY OF BRCA2 GENE

BRCA2 mutations are generally less common than BRCA1 mutations. Only in Iceland the situation is different where one single mutation in BRCA2 gene is responsible for almost all inherited breast and ovarian cancers (11). BRCA2 mutations are more frequent in families with at least one case of male breast cancer comparing to families with female breast and ovarian cancer. First

data shows that BRCA2 mutations are responsible for around 19% of familial male breast cancer (12). In the study conducted in Hungary, the country with highest male breast cancer mortality in continental Europe, the results have shown that 33% of patients carried BRCA2 germ line mutation but none of them reported family history of breast/ovarian cancer (13).

The estimated carrier frequency of BRCA2 germ line mutations among white individuals is similar to BRCA1 gene around 0.1%-0.3% and recently as low as 0.06% (14).

Similar to BRCA1 gene, mutation spectra studies of BRCA2 gene have revealed that the vast majority of mutations include deletions and insertions leading to frame shift and nonsense mutations (15). A large percentage of BRCA2 discovered germ line mutations belonging to this group (33%) are represented by two deletion mutations, 6174delT and 999del5. The 6174delT is found in about 8% of early onset breast cancer cases in Ashkenazi Jewish population (16). The other one, 999del5 has been commonly reported in early onset familial breast cancer cases from Iceland (17,11). This mutation represent the most dramatic example for the existence of the founder mutation where geographic and social factors influence the current population structure (18). A numerous group of detected mutations is marked as unclassified variants. Those are detected missense mutations with unknown influence on a gene function.

Analysis of 25 families with multiple cases of breast and ovarian cancer cases with detected BRCA2 mutations provided evidence for the existence of genotype-phenotype correlation. Mutations leading to the truncation of BRCA2 protein in families with highest risk for ovarian cancer were all localized in exon 11 (19).

The penetrance of BRCA2 mutations may be more variable than those of BRCA1. It looks like that BRCA2 belongs to a class of largely undiscovered but unquestionably existent low-penetrance cancer genes (20).

PHENOTYPE OF BRCA2 ASSOCIATED TUMORS

The phenotype of BRCA2 HBC is currently less investigated than that of BRCA1 HBC. The gene discovery is more recent, fewer families have been investigated and it looks like that its contribution to HBC may be lower.

BRCA2 HBC families are characterized with numerous cases of female and sometimes male breast carcinomas, and fewer ovarian carcinomas comparing to the BRCA1 HBC (21). Invasive ductal carcinoma accounts for 84-93% of cases carrying BRCA2 germ line mutation (22). Among BRCA2 HBC families in addition to breast carcinomas, a spectrum of different cancer types has been seen, prostate cancer and pancreatic cancer on the first place (23).

FUTURE PERSPECTIVES

Analysis of large breast cancer kindred from Iceland with detected 999del5 BRCA2 mutation showed that clustering of male breast cancer cases in one single branch of the pedigree is not likely to be a chance clustering but rather a result of the existence of genetic modifying factor augmenting the risk conferred by the present BRCA2 germ line mutation (11). The first evidence of a modifying genetic factor of BRCA2 in male breast cancer was provided recently, with the detection of duplication of 9p23-24 coexisting with BRCA2 germ line mutation of three brothers with breast carcinoma (24). One of the future perspectives is the identification of modifying genes influencing the penetrance of BRCA2 germ line mutations.

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