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BRCA 1 and BRCA 2: Role in Breast Cancer

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ABSTRACT

Breast cancer is the most common malignancy affecting women worldwide. It is one of the foremost reasons for death in women nowadays. BRCA1/2 associated breast cancers have distinctive features that vary from other cancers, comprising alterations in cellular molecules, pathological bases, biological behaviour, and a different prevention strategy. BRCA1 is a multifunctional protein that has been associated in many cellular processes, including genomic stability, the cell-cycle checkpoint, DNA-damage repair, apoptosis, and gene transcription. Germline mutations in the tumour-suppressor gene BRCA2 incline to breast cancer. BRCA2 plays a firm role in maintaining genome stability by regulating homologous recombination. BRCA2 more in recent times has been implicated in cytokinesis, the final step of cell division, but the molecular basis for these remains unknown.

KEYWORDS: Breast Cancer, BRCA1, BRCA2, Mutation.

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INTRODUCTION

Breast cancer is the utmost common cancer affecting women worldwide. Even though many risks and prognostic factors of breast cancer have been recognized, and several biomarkers have been linked to breast cancer. In the development and progress of breast cancer recently, evidence has rapidly accumulated on the potential role of multiple metabolic disorders. Patients who receive genetic defects in BRCA1 and BRCA2 have a bigger lifetime risk of emerging breast cancer. However, the exact mechanism by which loss of BRCA1 affects specific tissues in humans is blurred. Germline mutations of the BRCA1 tumour suppressor gene are the main cause of familial breast cancer. BRCA1 plays serious roles in a number of varied cellular processes that ensure genome integrity and the bigger risk of breast and ovarian cancer caused by mutation of BRCA1 has been attributed to increased genomic instability. To protect genome, cells have developed a defensive mechanism, called the DNA damage response, to synchronize multiple cellular responses including DNA repair, cell cycle checkpoint regulation, transcription, senescence or apoptosis etc., to respond to genotoxic stress[1,2,3,4].

Latest developments in molecular biology, enable researchers to understand the idea of breast cancer in an exceedingly far better manner and show that hereditary breast cancer could outcome from mutations on many specific gene loci as well as BRCA1, BRCA2, p53, ATM and PTEN. These genes are tumour suppressor genes and while their functions are various, they're all concerned with the preservation of genomic stability once DNA damage. Mutations that harm the function of those genes could adversely have an effect on the way within which DNA damage is processed. It's possible that the chance of carcinoma development is accumulated through this mechanism. What is more, there are varied predispositions, like the androgenic hormone receptor gene (AR) and also the HNPCC that will even be concerned, however, a lot of studies are needed so as to understand the extent of their involvement in breast cancer. In this paper, we have a tendency to offer knowledge of the general function of the tumour suppressor genes indicated above. We also review credible mutations of these genes and their significance to breast cancer development. Moreover, we discuss estrogen genes and estrogen receptor genes that will be concerned about breast cancer development, as indicated within the recent studies.

BRCA1 AND BRCA2- OVERVIEW

The identification of the BRCA1 and BRCA2 genes (Fig. 1) will have vast significance in furthering our understanding of breast pathogenesis. BRCA1 and BRCA2 are responsible for 80-90% of all familial breast cancer[5]. The BRCA1 gene, mapped to chromosome 17q21, is a large one (Fig. 1). It spans 100 kb of genomic DNA and encodes a protein of 1863 amino acids. This gene, transcribed in several tissues, was found to be most abundantly expressed in the thymus, testis, breast

and ovary. It is known that the gene does not have homology with other genes except for the zinc finger domain at the N-terminus and a heptad repeat element in the middle of the protein, which might enhance dimerization. BRCA1 product is involved in DNA repair, transcriptional transactivation, apoptosis and cell cycle control[6]. How does BRCA1 perform these functions? Experiments conducted so far indicate that wild type BRCA1 protein binds to a number of cellular proteins, including DNA repair protein Rad 51, tumour suppressor p53, RNA polymerase II holoenzyme, RNA helicase A, CtBP-interacting protein, c-myc, BRCA1- associated RING domain protein (BARD1), BRCA2 protein, etc. These proteins probably mediate functions of BRCA1. Therefore, mutations in BRCA1 may affect the composition of these complexes and dysregulation of their functions may eventually result in the development of malignancy[7]. BRCA2 undergoes differential splicing, giving rise to a novel variant protein, BRCA2a, lacking putative transcriptional activation domain. Both BRCA2 and BRCA2a are expressed at high levels in the thymus and testis but at moderate levels in the mammary gland and prostate, suggesting that BRCA2 and BRCA2a play a role in the development and differentiation of these tissues[8]. Mutations in BRCA1 and BRCA2 appear to confer essentially similar risks of female breast cancer. The risk of ovarian cancer is lower in those with BRCA2 mutations, though the risk of male breast cancer in those with a BRCA2 mutation is substantially higher. The risk of other cancers, including laryngeal and prostate, may also be elevated in carriers of BRCA2. Loss of heterozygosity involving the BRCA2 locus at 13q12 (Fig. 1), but not the RB1 locus at 13q14, has been observed in sporadic breast, pancreatic, head and neck, and other cancers, suggesting that there is a somatically mutated tumour suppressor gene in the vicinity of BRCA2. BRCA2 is a strong candidate for this gene. Finally, the predicted sequence of the BRCA2 protein has shed little light on its function, though preliminary studies have shown that it bears a very weak similarity to the BRCA1 protein over a restricted region of the sequence.

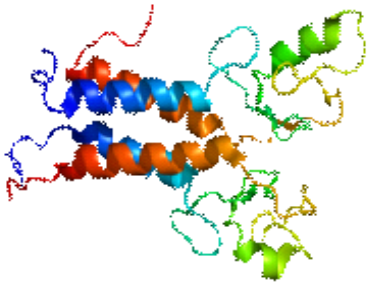
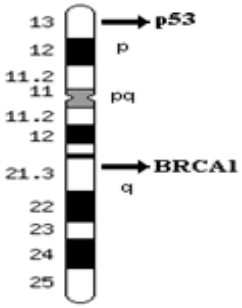
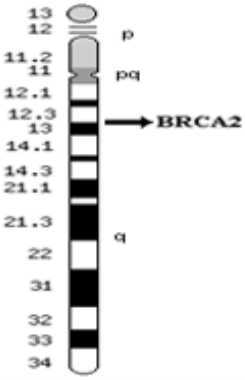
Gene and 3D protein structure	Chromosome location	Function	Primary tumor	Syndrome
BRCA1 	17q21 	Interacts with Rad 51 protein; Repair of double-strand breaks; involved also in transcriptional transactivation apoptosis and cell cycle control	Breast cancer	Familial breast cancer
BRCA2 	13q12 	Interacts with Rad 51 protein; repair of double-strand breaks; Also has a role in transcriptional regulation.	Breast cancer	Familial Breast cancer 2

Figure 1. BRCA1 And BRCA2 Genes, 3D Structure, Chromosomal Location and Primary Function.

BRCA1 has two important structural motifs, including a highly conserved amino-terminal RING finger motif and tandem BRCT motifs at its C-terminus[9,10]. The RING finger motif confers BRCA1 E3 ubiquitin ligase activity, one of the intriguing aspects of BRCA1 function, regulating activity, stability and distribution of target molecules[11]. The BRCT region of BRCA1 is essential for its DNA repair, transcriptional regulation and tumour suppressor functions[12]. Germline mutations in BRCA1 were often seen in the two regions[13], suggesting that the RING finger and BRCT motifs play an important role in the development of breast and ovarian cancers.

BRCA1

The inheritance of germ-line mutations in autosomal dominant susceptibility genes appears to be responsible for 5–10% of all breast cancer cases[14]. Because most breast tumours arising in patients with germ-line BRCA2 mutations have been found to exhibit loss of the wild-type BRCA2 allele, this gene is believed to function as a tumour suppressor. Nevertheless, little is currently known about the function or regulation of this gene. The dramatically elevated risks of breast cancer observed in women carrying germ-line mutations in either of the familial breast cancer susceptibility genes, BRCA1 or BRCA2, suggest that these genes play important roles in the regulation of

mammary epithelial cell growth. This suggestion is consistent with the observation that the murine homologue of BRCA1 is widely expressed in proliferating and differentiating cell types in the mouse during embryonic and mammary gland development and in adult tissues[15]. Specifically, BRCA2 is expressed at high levels in rapidly proliferating and differentiating cellular compartments, including those in the breast, such as terminal end buds during puberty and differentiating alveoli during pregnancy. Nevertheless, despite the intriguing similarities in the developmental regulation of BRCA1 and BRCA2expression in the mammary gland, the basis for this similarity is currently unknown, as is the extent to which these cancer susceptibility genes may be regulated by or involved in the processes of proliferation and differentiation in no transformed mammary epithelial cells.

BRCA1 was first located to chromosome 17 via a genetic linkage analysis in 23 early-onset breast cancer families [16], and was cloned and isolated in 1994 [9]. Further research had localized it to 17q21 with a length of 100 kb. BRCA1 has 24 exons, including 2 non-translating exons, encoding a protein of 1 863 amino acids, which is characterized by a zinc-binding RING finger domain at the amino terminus and BRCA1 carboxyl-terminal (BRCT) domain at the carboxyl terminus. BRCA1 is classified as a tumour suppressor gene and plays an important role in the surveillance of cell cycle and repair of DNA damage. Evidence shows that BRCA1 is phosphorylated by the checkpoint kinase ataxia telangiectasia mutated (ATM) protein after ionizing radiation[17]. Mediator of DNA damage checkpoint protein 1 (MDC1) can regulate BRCA1 to the sites of DNA lesions and phosphorylate it through ATM-dependent pathways[18]. After activation, BRCA1 can bind to p53, RAD50-MRE11-NBS1 (R-M-N) complex and RAD51, conducting homologous recombination or non-homologous end-joining (NHEJ) which is of great importance in DNA damage repair. The zinc-binding RING-finger domain of BRCA1 can interact with BRCA1 associated RING domain 1 (BARD1) forming a heterodimeric complex that has ubiquitin ligase activity and the complex itself may be involved in DNA damage repair.

MUTATIONS IN BRCA1

It accounts for approximately 45% of families with a high incidence of breast cancer and for the majority of families with a high incidence of both breast and ovarian cancer. Several lines of evidence indicate that BRCA1 is a tumour suppressor, but a role as a negative regulator of cell proliferation is yet to be unambiguously demonstrated. BRCA1-linked tumours arising in carriers of germ-line mutations display loss of heterozygosity in the BRCA1 locus with retention of the mutant allele. BRCA1 induces the expression of the cyclin-dependent kinase inhibitor p21Waf1/CiP1, causing cell-cycle arrest3. Conversely, inhibition of BRCA1 expression with antisense oligonucleotides results in accelerated proliferation in a mammary epithelial cell line4. It is still not clear whether these effects of BRCA1 on cell proliferation correspond to a physiological function or

represent a response to abnormal levels of the protein induced by experimental conditions. Human BRCA1 codes for an 1863- amino-acid nuclear protein (Fig. 1) with no detectable similarity to known proteins, with the exception of a RING-finger domain located in the N terminus and two BRCT (BRCA1 C-terminal) domains in tandem (aa 1653–1736 and aa 1760–1855)[9,5]. The BRCT is a globular domain found in proteins involved in repair and cell-cycle control. Most of the documented cancer-associated mutations cause truncations of the C-terminal region, a highly evolutionarily conserved region of the protein comprising the BRCT domains, underscoring the importance of this region for function. BRCA1 has been found in large complexes that contain proteins involved in DNA repair and human cells lacking BRCA1 display high sensitivity to g-irradiation[19]. In addition, BRCA1 seems to be required for efficient homologous recombination. To date, the evidence implicating BRCA1 in a variety of DNA-repair processes are based largely on genetic experiments and do not reveal by which mechanism BRCA1 acts. Although many scenarios remain possible at this stage, it is plausible that these effects are indirectly mediated through transcription activation.

Similarly, the C-terminal region of BRCA1 was shown to bind to the histone deacetylases HDAC1 and HDAC2. Two independent approaches to identify BRCA1-binding proteins, one using the yeast two-hybrid system and the other using the Sos-recruitment system (a variation of the two-hybrid system commonly used when the proteins to be tested display intrinsic transcriptional activity), resulted in the isolation of CtIP (CtBP-interacting protein), a co-repressor for different cellular transcription factor. Cancer-associated mutations in BRCA1 abolished binding to CtIP, as did DNA damage. Of particular importance to breast and ovary carcinogenesis are preliminary reports that BRCA1 modulates estrogen receptor a transcription36, but further studies are needed to determine the physiological relevance of this modulation. It is possible that BRCA1 also regulates promoter selectivity of ER a target genes.

BRCA2

In 1995, a second gene termed BRCA2 was found related to hereditary breast cancer[20]. It covers about 70 kb of genomic sequence in 13q12, encoding a protein of 3 418 amino acids. The coding region of BRCA2 is composed of 27 exons with a non-translating exon. However, the gene sequence of BRCA2 bears no obvious homology to any known gene including BRCA1, and the protein contains no defined functional domains[20]. BRCA2 can bind with BRCA1, participating in DNA damage response pathway associated with the activation of homologous recombination and double-strand break repair[21]. For their key role in maintaining genomic integrity and supervising cell cycle, mutations in BRCA1 and BRCA2 are found strongly related to hereditary breast cancers. However, the types of mutation differ in distribution by ethnicity and geographic location.

Heterozygous germline mutations in BRCA2 (Breast Cancer 2, early onset) are associated with an increased risk of developing breast and ovarian cancer[22]. BRCA2, a large 384 kDa protein, plays a key role in the repair of DNA damage by means of homologous recombination. It regulates the function of the Rad51 recombinase and is required for the formation of nuclear Rad51 foci[23]. Cells that are deficient in BRCA2 are sensitive to ionizing radiation and accumulate chromosomal rearrangements[24]. BRCA2-deficient cells not only show structural chromosome aberrations but also exhibit an increase in the chromosome and centrosome number[24]. Both latter defects point towards a potential role for BRCA2 in the regulation of mitosis. Consistent with this notion, analysis of murine and human cells lacking BRCA2 function revealed defects in cytokinesis, the final stage of cell division[25]. Cytokinesis is the process that partitions segregated sister genomes to nascent daughter cells at anaphase by virtue of ingression of a cleavage furrow[26]. The process is driven by the constriction of a membrane-coupled actomyosin ring and completed by membrane fusion (abscission) yielding two distinct daughter cells. The formation and positioning of the cleavage furrow rely on the spindle midzone, a microtubule-based structure that forms at anaphase between the segregating sister chromatids and concentrates a number of cytokinesis regulators[26]. Interestingly, BRCA2 was reported to accumulate at the spindle midzone[25,27]. Although it is currently unknown whether defects in cell division caused by mutations in BRCA2 contribute to tumorigenesis, a number of recent studies have suggested that tetraploidy caused by cell-division failure might be a transient intermediate on the road to aneuploidy and cancer[28]. The sociomedical importance of BRCA2 underscores the need to understand its cellular functions. At present, the molecular basis of the role of BRCA2 in cell division is unknown. It is furthermore unclear whether the cytokinetic defects observed upon loss of BRCA2 reflect the direct involvement of the protein in the process or whether they are an indirect consequence of chromosomal lesions and defective DNA repair. To address these questions, we examined the function and localization of human BRCA2 during cell division.

FUTURE PERSPECTIVES

Literature about breast cancer dates back to the second half of the 19th century. Due to the lack of genetic knowledge, studies until the 20th century were on the epidemiological level. Until 1990, there was no progress in the characterization of breast cancer genes. In the early 1990s, some investigators showed certain mutations of the p53 gene involved in the development of breast cancer. Afterwards, BRCA1 and BRCA2 genes were discovered to play a role in breast cancer. Since then, other genes and chromosomal abnormalities have been found to participate in the carcinogenesis of breast tissues. Thus, information regarding the formation of breast cancer at the genetic level has been obtained. In breast tissue, the hormone-sensitive cells in the terminal duct-lobular unit contain

the stem cells that generate the lactating lobules. These cells are responsive to estrogen and progesterone, which provide signals for growth during the menstrual cycle and elicit proliferation during pregnancy. Should the individual carry a germline or somatic mutation in tumour suppressor genes, the stem cells in the terminal ductal-lobular unit are predisposed to malignancy, but these cells are quiescent in prepubertal life, and no tumour can form. When these cells are subjected to hormone stimulation during puberty, their DNA is replicated to permit cell proliferation. However, if there is a genetic defect in p53 or in the other genes, the control and regulation of replication cannot be carried out in a proper manner. Therefore, cells start proliferating in an uncontrolled way, thus causing instability and activation of proto-oncogenes. Some oncogenes also initiate gene amplifications (erbB2, c-myc, int-2), leading to tumorigenesis. Although the activation of oncogenes has clear relevance in selected breast cancer cases, a more common finding in breast cancer cells is a mutation in one or more tumour suppressor genes. As a class, these genes function to maintain genomic integrity and help prevent the propagation of damaged DNA. Aberration in many tumour suppressor genes directly affects cellular susceptibility to DNA damage and cellular capacity DNA damage repair. Others recognize damaged DNA and promote cell cycle arrest, allowing for repair of damage before DNA synthesis and mitosis commence. Finally, tumour suppressor gene products may also inhibit the propagation of damaged DNA by inducing apoptotic cellular death.

BRCA1 contacts the RNA polymerase II holoenzyme components p300/CBP, RNA helicase A, RPB10a and RPB2. The fact that RPB10a is a common component of all three RNA polymerases raises the possibility that BRCA1 might also regulate pol I and pol III transcription, and it will be important to see if BRCA1 is also present in these complexes. However, to date, no one has been able to demonstrate sequence-specific binding to DNA by BRCA1, although several instances where BRCA1 functionally interacts with other DNA-binding proteins have been reported. Alternatively, BRCA1 might only interact with holoenzyme complexes engaged in transcription. The in vitro transcription reconstitution assays can be used to distinguish between these possibilities. It might be feasible to immunodeplete BRCA1- containing holoenzyme complexes and compare the activities of different holoenzyme preparations. Are BRCA1-target genes the same in different tissues? Because tissue specificity for tumour formation cannot be explained by expression patterns (as BRCA1 is ubiquitously expressed), it is plausible that BRCA1 might be required for a subset of genes that are highly transcribed in certain tissues such as breast and ovary. Therefore, lack of functional BRCA1 would prime cells in these tissues to transformation. In light of the data on the involvement of BRCA1 in TCR, this idea is particularly appealing. It will be crucial to determining which biochemical step in TCR requires BRCA1. The evidence for the role of BRCA1 in transcriptional regulation can be summarized as follows: (i) the C terminus of BRCA1 acts as a transcriptional

activation domain when fused to a heterologous DNA-binding domain; (ii) BRCA1 can be found in complex with the RNA polymerase II (core and holoenzyme); (iii) ectopic expression of BRCA1 induces transcription from a variety of different promoters; and (iv) several BRCA1-interacting proteins have well-characterized roles in transcription. The strong correlation of cancer-associated mutations and its loss of function phenotype in the experiments described strengthening the idea that the role of BRCA1 in transcription is physiologically relevant during the development of the disease. Many questions remain unanswered, but the biochemical and genetic approaches discussed here form the basis to attribute a definite biological function for BRCA1.

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