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Molecular Genetics of Breast Cancer

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Abstract: Recent advances in molecular biology enable researchers to understand the basis of breast cancer much better and show that herediteny breast cancer may result from mutations on several specific gene loci including BRCA1, BRCA2, p53, ATM and PTEN. These genes are tumor supressor genes and although their functions are different, they are all involved in the maintenance of genomic stability after DNA damage. Mutations that impair the function of these genes may adversely affect the manner in which DNA damage is processed. It is likely that the risk of breast cancer development is increased through this mechanism. Moreover, there are several predispositions, such as the androgen receptor gene (AR) and the HNPCC, that may also be involved, but further studies are required in order to understand the extent of their involvement in breast cancer. In this paper, we supply data on the general function of the tumor supressor genes indicated above. We also review probable mutations of these genes and their relevance to breast cancer development. Furthermore, we discuss estrogen genes and estrogen receptor genes that may be involved in breast cancer development, as indicated in the recent studies.

Key Words: BRCA1, BRCA2, p53, ATM, PTEN and Estrogen

Introduction

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 1990's, 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 (1). 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 tumor supressor genes, the stem cells in the terminal ductal-lobular unit are predisposed to malignancy, but these cells are quiescent in prepubertal life, and no tumor 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 (erbB$_2$, c-myc, int-2), leading to tumorogenesis (2). 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 tumor suppressor genes. As a class, these genes function to maintain genomic integrity and help prevent the propagation of damaged DNA. Aberration in many tumor 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, tumor suppressor gene products may also inhibit propagation of damaged DNA by inducing apoptotic cellular death (3).

Although it has been demonstrated that mutations of p53, BRCA1 and BRCA2 can lead to increased breast cancer risk, the percentage of these mutations is comparatively low in breast cancer cases (only 6% to 8% of the total United States breast cancer population) (4, 5). In addition, these genes are not found in all cases of familial breast cancer. Therefore, further studies should be carried out to isolate germline genetic etiologies. In this respect, studies are being conducted to discover new genes related to breast cancer, and in particular it has been indicated that the involvement of estrogen in breast cancer may be much deeper than was originally thought.

BRCA1 and BRCA2

The identification of the BRCA1 and BRCA2 genes will have vast significance in furthering our understanding of breast pathogenesis. These two genes are tumor supressor genes shown to be involved not only in breast cancer but also in ovarian and prostate cancers (Table). BRCA1 and BRCA2 are responsible for 80-90% of all familial breast cancer (6). 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 (Table). 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 (1,7-9). 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, tumor supressor 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 disregulation of their functions may eventually result in the devolopment of malignancy (Fig. 2) (10).

It was also found that BRCA1 has two variants, BRCA1a and BRCA1b, which are phosphoproteins containing phosphotyrosine (11). These proteins are

Table.   Summary of genes involved in breast cancer.

| Cloned gene	Chromosome location	Proposed function of gene product	Primary tumor	Associated cancers or traits	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	Ovarian 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	Male breast cancer, cancer, pancreatic, ?others (for example, ovarian)	Familial breast cancer 2
p53	17p13.1	Transcription factor; response to DNA damage and stress; apoptosis	Sarcomas, breast cancer	Brain tumors, leukemia	Li-Fraumeni Syndrome
ATM	11q22	DNA repair, induction of p53, phosphorilation of c-Abl and BRCA1	Lymphoma	Cerebellar ataxia, immunodeficiency, breast cancer in heterozygotes	Ataxia telengiectasia
PTEN	10q23	Control PIP3 pathway in cell growth, keep cell population in check.	Glioma	Menengiomas, prostate cancer, breast cancer	Cowden's disease
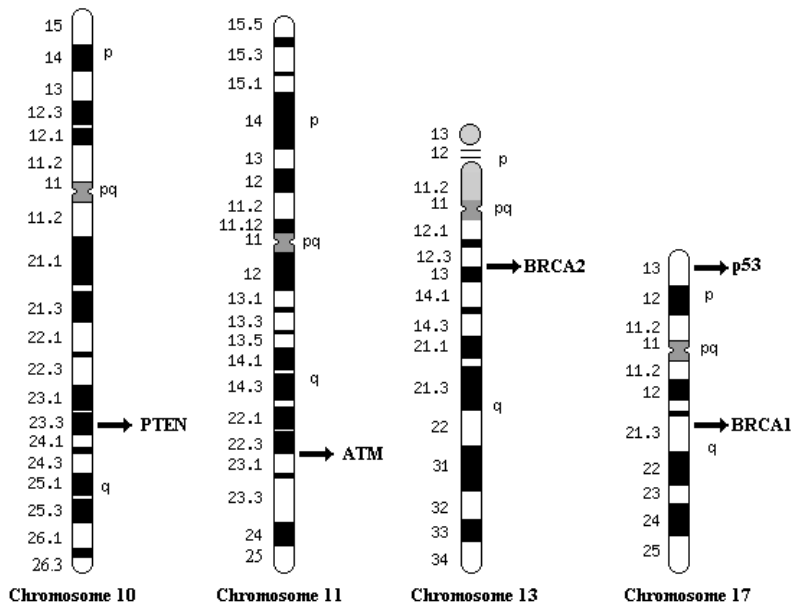
Fig. 1.   Ideogram of human chromosomes 10, 11,13 and 17 showing the position of PTEN, ATM, BRCA2, BRCA1 and p53 respectively.
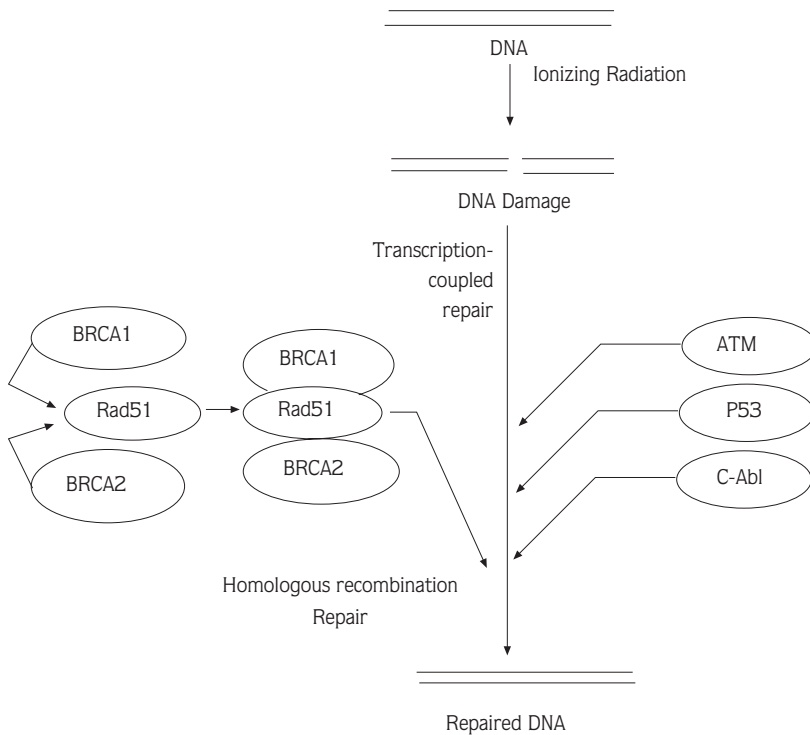

Fig. 2.   A schematic diagram of possible pathways by which BRCA genes affect DNA damage repair.

associated with E2F, cyclins and cyclin dependent kinases. These findings suggest that BRCA1 could be an important negative regulator of cell cycle that functions through interaction with E2F transcriptional factors and phosphorylation by cyclins/cdk complexes, with zinc ring finger functioning as a major protein-protein interaction domain. But these interactions are observed *in vitro*. If they are also observed *in vivo*, then it may be that the lack or impaired binding of the disrupted BRCA1 proteins to E2F and cyclins/CDKs in patients with mutations in the zinc finger domain could deprive the cell of an important mechanism for braking cell proliferation, leading to the

3

development of breast and ovarian cancers. BRCA1a and BRCA1b also interact with p53 *in vitro* and in vivo. These results demonstrate the presence of a second p53 interaction domain in BRCA1 proteins and suggest that BRCA1a and BRCA1b proteins, like BRCA1, function as p53 co-activators (12).

A great variety of germline mutations are thought to result in the synthesis of a truncated BRCA1 protein. Although most mutations have been identified in only one or two families, a limited number of them have been seen recurrently (13). The nature of mutations are displayed and it was shown that these mutations form allelic variants of BRCA1 (Fig 3). Of these, the most common is the variant BRCA1, 2-BP DEL, 185AG, which includes a deletion of the normal sequence TTA GAG of codons 22-23 in exon 3. These mutations alter the reading frame of the mRNA and cause a premature termination at position 39 (14). Of particular interest, frameshift mutations at codon 185 of BRCA1 have been identified in more than 20 Jewish families with familial breast or ovarian cancer. A recent population survey of Ashkenazi Jews, chosen without regard to family history of cancer, has demonstrated that about 1% carry the 185delAG mutation (15). Thus, as suggested by Collins, familial breast and ovarian cancer attributable to the 185delAG mutation is potentially the most common serious single-gene disorder yet identified in any population group (16).

Based on epidemiologic studies, the life-long risks of breast cancer and ovarian cancer in individuals carrying a mutant BRCA1 allele have been estimated at 85% and 50% respectively (13). BRCA1 mutations have been observed in roughly 10% to 15% of women who had developed breast cancer before the age of 35. In Jewish women who developed breast cancer before the age of 40, BRCA1 mutations were seen in over 20% (17).

Based on the fact that the LOH of the BRCA1 locus was seen in 50% of unselected breast tumors and in 60% to 65% of unselected ovarian tumors (18), the BRCA1 protein was hypothesized to play an important role in the development of sporadic breast and ovarian cancers. Surprisingly, very few tumors have been found to harbor detectable somatic point mutations in BRCA1 (18). Hence, these findings have raised questions regarding the role of the BRCA1 gene in sporadic breast and ovarian cancers. However, two recent reports have suggested a role for the BRCA1 gene in sporadic tumors. Somatic BRCA1 mutations have been identified in at least a few sporadic ovarian carcinomas (19). Moreover, a recent study has hinted that the function of the BRCA1 protein may indeed be altered in the majority of breast and ovarian cancers. Specifically, it was found that the BRCA1 protein normally localized in the cytoplasm in the majority of breast and ovarian carcinoma cell lines and primary tumors evaluated (20). These findings suggest that
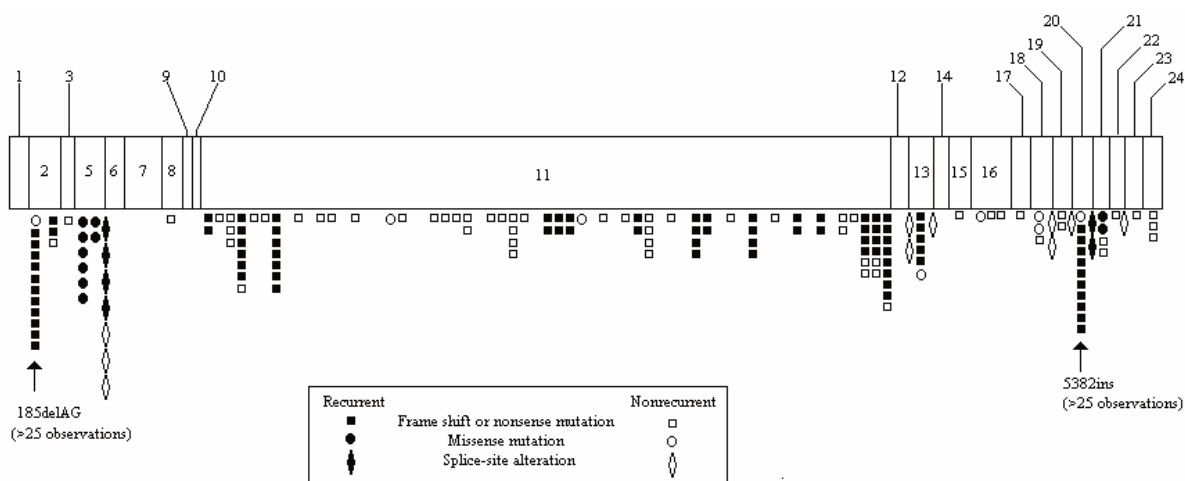

Fig. 3.   Germline mutations reported in the BRCA1 gene predominantly from families with high incidence of breast and/or ovarian cancer. The numbers correspond to BRCA1 exons; there is no exon 4. The nature of the mutations and whether they have been seen in more than one apparently unrelated individial (i.e., recurrent) are indicated. (From Collins, 1996, with permission).

BRCA1 abnormalities, the nature of most of which is as yet unknown, may play an important role in many sporadic as well as familial breast and ovarian cancers.

On the other hand, functions of BRCA1 have been observed in various human epithelial cancer cell types and mouse fibroblast and do not explain the association of BRCA1 mutations with specific tumor types, such as breast cancer. Estrogen stimulation of the mammary epithelia is thought to be a major factor in promoting the development of breast cancer, probably through the expansion of previously initiated mammary epithelial cell clones (21). Thus, S. Fun and his colleagues hypothesized that the BRCA1 protein might function, in part, to regulate the cellular response to estrogen. To assess the effect of BRCA1 on estrogen response, they measured the ability of the BRCA1 gene to modulate the transcriptional activity of ER-α in transient transfection assays. They found that the wild-type BRCA1 gene (wtBRCA1) inhibits ER-α signaling in breast cancer cell line T47D, in two other human breast cancer cell lines (MCF7 and MDA-MB-231), and in two human prostate cancer cell lines (LNCaP and TsuPr-1) . However, three human cervical cancer cell lines showed relatively weak inhibition of ER-α signaling by BRCA1. But these observations are hypothetical and to establish the validity of these hypotheses, it is necessary to confirm the ability of BRCA1 to regulate the ER-α response in *in vivo* models (22).

While germline mutations in the BRCA1 gene account for upwards of half of the families in which many members are affected by breast cancer, other cases of familial breast cancer susceptibility have been attributed to mutations of another highly penetrant autosomal dominant susceptibility gene termed BRCA2. The BRCA2 gene was mapped to chromosome 13q12-13 in 1994 and was recently characterized. BRCA2 is larger than BRCA1, with a coding sequence of 10 254 base pairs encoding 3418 amino acids and 26 coding exons (23-26). The BRCA2 protein, like BRCA1, plays a role in transcriptional regulation and DNA repair (Fig. 2). It has been shown that BRCA2 has transcriptional activation potential conferred by its amino terminal third exon. Published results (1998) present evidence that BRCA2 has intrinsic HAT activity, which maps to the amino-terminal region of BRCA2. In this study it is also demonstrated that BRCA2 proteins acetylate primarily H3 and H4 of the free histones. These observations suggest that the HAT activity of BRCA2 may play an important role in the regulation of transcription and tumor supressor function (27). But, in another study by Fuks and his co-workers, no evidence was found in support of an intrinsic HAT activity in the BRCA2 amino terminus. They also show that BRCA2 interacts with a transcriptional co-activator protein, P/CAF, which possesses histone acetyltransferase activity. The interaction with P/CAF is demonstrated *in vitro* as well as in vivo and is shown to be mediated by residues 290-453 of BRCA2. Thus, the Fuks team suggests that one mechanism by which BRCA2 regulates transcription may be through the recruitment of the histone modifying activity of the P/CAF (28).

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 (29).

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 (24). 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 tumor 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 sequence (24). The significance of this finding is unclear.

P53

p53 mutations are very prevalent in a wide spectrum of human cancers (Table). Indeed, p53 is believed to be one of the most commonly mutated genes in human cancer (30). The p53 gene is located on chromosome 17p13.1 (Fig. 1), contains 393 codons and codes for a

nuclear protein of 53 000 D (53 kD). A region with transcription activating (ACT) properties is located at the N-terminal end of its gene product. The HSP domain (coded for codons 13-29) of mutant p53 can bind to heat-shock proteins. A serine residue at position 315 can become phosphorylated by the product of the CDC2 gene, a gene in cell-cycle regulation. The p53 protein is a transcription factor and plays an important role in regulating growth in damaged cells as well (31).

As indicated above, mutations in the p53 gene are the most frequent genetic changes in cells in different malignant tumors of man. Mutations in the p53 gene frequently occur (in 12-13% of all tumors) in codons 175, 248 and 273 (32). Detailed characterization of the mutations present in p53 has revealed that the pattern of DNA base substitutions is distinctly different in different types of cancers.

In primary breast cancers, mutations of the p53 tumor suppressor gene are common and lead to loss of growth-suppressive properties and poor outcome (33). Approximately 20% to 40% of human breast cancers have mutations in the p53 gene (34). Individuals with a p53 germline mutation (Li-Fraumeni syndrome) have very high risks of breast cancer and other malignancies (35). Cancer risks in individuals with a germline p53 mutation are estimated to be 50% by the age of 30 and 90% by the age of 70 (36). In a study of 231 patients with germline p53 mutations, breast cancer was the most common malignancy (37). Smith and his colleagues have indicated that inheritance of germline mutant alleles of BRCA1 and BRCA2 confers a markedly increased risk of breast cancer. In their previously published study, it was reported that p53 mutations have a higher incidence in these tumors. They have characterized these new p53 mutants. The rarity of these mutants in human cancer and their multiple occurrence in BRCA-associated breast tumors suggest that these novel p53 mutants are selected during malignant progression in the unique genetic background of BRCA1 and BRCA2 associated tumors (38). Breast cancers occurring in BRCA1 mutation carriers had significantly higher levels of p53 expression, including the preinvasive (carcinoma *in situ*) stage of disease, compared with cancers occurring in BRCA2 mutation carriers or women with no detectable germline mutation (39). Direct p53 mutation analyses revealed mutations in 18% of all of the early-onset breast cancers within the study and included rare insertion and deletion

mutations in cancers from BRCA1 mutation carriers. Likewise, several studies indicate that the short arm of chromosome 17 is one of the most frequently altered regions in sporadic breast carcinomas (45-60%) (40). In this regard, the study by Liscia's team is further evidence of the presence, within the region, of at least a second tumor suppressor gene distal to p53, which might be targeted by deletions.

On the other hand, it was demonstrated that 17β-estradiol (E2) induces p53 protein expression in breast cancer cells. Direct effects of E2 on the expression of the p53 gene are not known but it is clear that the steroid is a potent regulator of c-Myc transcription. In a study published by Hard's team, the ability of E2 and antiestrogens has been examined to regulate the P1 promoter of the p53 gene which contains a c-Myc responsive element. In the end, they demonstrate both ER-mediated and ER-independent regulation of c-Myc and P1 promoter of the p53 gene, and show differential effects of the two classes of antiestrogens in their ability to induce the P1 promoter of the p53 gene in breast cancer (41).

Recently, two p53-related genes, p63 and p73, were discovered. As it might be predicted, both genes encode proteins with transactivation, DNA binding and tetramerization domains, and they share considerable homology with p53 (42). Ectopic p73 expression can activate a broad subset of p53 responsive genes, induce apoptosis, and act as a growth suppressor. Many of p53's targets can be transactivated by some isoforms of p63 and p73. Some forms also induce cell-cycle arrest and apoptosis. This shows that some p53 independent cellular responses are given by these p53 relatives (42). It was also found that p73 is a target of the non-receptor tyrosine kinase c-Abl in response to DNA damage (Fig. 4) (43-45). Zaika and his colleagues analyzed 77 invasive breast cancers and 7 breast cancer cell lines for p73 mRNA expression levels, allelic origin, intragenic mutations, and COOH-terminal splice variants. They found that p73 is overexpressed in breast cancer, and also found six different COOH-terminal splice variants. They confirm the previously described variants gamma and delta in breast tissue and describe two novel isoforms, p73 epsilon and phi, thereby further enlarging the combinative possibilities. In conclusion, their in vivo data show that p73 does not play a role as a classic Knudson-type tumor suppressor in breast cancer (46).
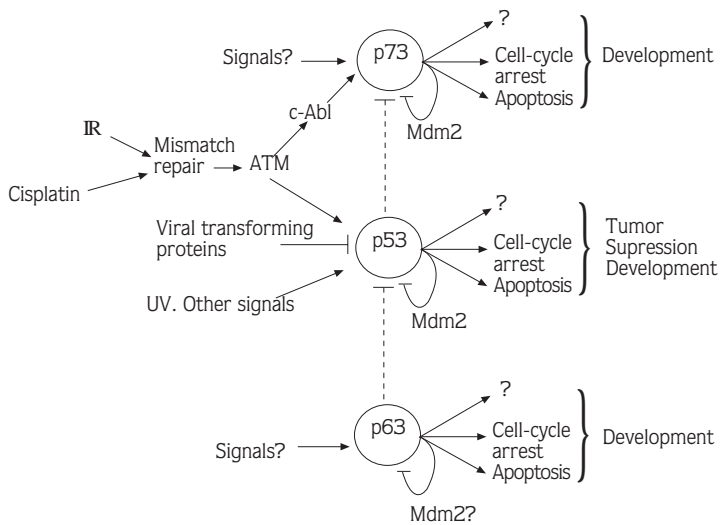
Fig. 4. Regulatory targets and downstream affecting p53 and related proteins p63 and p73. Gong et al., Agami et al., and Yuan et al.

In the other study, related p73 has been elucidated the functional significance of the gene in the oncogenesis of breast cancer. But observations regarding this study suggest that allelic loss, expression levels and mutations of the p73 gene may not contribute to the oncogenesis of primary breast cancers (33).

ATM

In recent studies, it has been shown that the gene for ataxia telangiectasia (AT), called ATM, may play a role in the development of breast cancer (Table). The ATM gene has been recently cloned and characterized. This gene was identified by positional cloning on chromosome 11q22-23 (Fig. 1) (48). The gene codes for a large RNA transcript of 13,000 bp, of which a 3 500 amino acid protein is translated. ATM itself covers 150 kb, spreading over 64 exons (44). In another study, it was shown that the ATM gene product, a component of the DNA-damage checkpoint interacts with and phophorylates c-Abl (Fig. 4) (49-51). The ATM protein is a widely expressed member of the family of protein kinases with similarities to phosphotidylinositol 3-kinases (42). The amino acid sequence has revealed the existence at the carboxyterminal end of the protein, of a domain presenting homology to PI-3 kinase. This characteristic has allowed the description of a new family of nuclear proteins, in yeast, drosophila and humans, functionally involved in DNA damage signaling (48). It is interesting to note that a vast majority of mutations described in AT patients lead to the truncation of the protein and consequently to the elimination of the PI-3K domain.

Thus, loss of ATM function in human and mouse cells causes defects in DNA repair and cell-cycle checkpoint control and this leads to susceptibility to cancer (42). Furthermore, after ionizing radiation, c-Abl also interacts with DNA-PK and DNA-end binding protein ku (52). DNA-PK can induce c-Abl activity and also appears to be an upstream mediator of p53 activity (53). This regulatory interaction of ATM, c-Abl, DNA-PK and p53 appears to be one critical link of DNA damage repair and cell cycle regulation. There is strong evidence that a key downstream target of ATM is p53, which is phosphorylated and stabilized by ATM in response to DNA damage (54-56).

Patients who are homozygous for ataxia telangiectasia (AT) have an exceptionally high incidence of cancer. An important question linked to AT mutation concerns the cancer risk associated with heterozygous mutations. It is well established that AT patients, homozygous for the mutation, present a 100-200 fold increased risk of cancer. Epidemiological studies have described a 3-5 fold increase risk of cancer (particularly breast cancer in women) associated with the heterozygous mutation. Knowing that the incidence of the heterozygotes can be estimated to range from 0.5 to 1% in the general population, this question is of great importance in terms of public health (48).

AT heterozygotes (estimated to be in 1% of the population) do not show any of the major symptoms of the disease, though there is good evidence that they have an underlying cellular radiosensitivity, but to a lesser

extent than observed in AT homozygotes (57). These observations, together with earlier epidemiological studies, reveal an increased incidence of mortality from cancer among blood relations of patients with ataxia telangiectasia, with the greatest relative risk for breast cancer in female relatives of patients (58).

An association between the incidence of breast cancer and AT heterozygosity was also revealed in two separate but smaller studies (59-60). Based on an independent assessment of all these data, the relative risk of breast cancer in AT heterozygotes was estimated to be 3.9, with AT carriers representing 3.8% of all cases (61).

With knowledge of the sequence of the ATM gene, FitzGerald and his co-workers detected heterozygous mutations in 2/202 healthy women (1%) with no personal history of cancer (62). The frequency of 1% is consistent with that predicted from epidemiological studies (58). When patients with early onset breast cancer (<40 years) were screened, 2/410 (0.5%) showed mutations in the ATM gene. FitzGerald and his co-workers therefore concluded that "heterozygous ATM mutations do not confer genetic predisposition to early onset breast cancer." On the other hand, a recent study by Athma and his colleagues using molecular genotyping suggested that AT heterozygotes are predisposed to breast cancer (63). Among 33 women with breast cancer, 25 were AT heterozygotes, compared with an expected 15. For patients with earlier onset disease (<60 years) the odds ratio was 2.9 (21 cases) and for older patients it was 6.4 (12 cases). Based on these relative risks, the authors calculated that 6.6% of all cases of breast cancer in America occur in AT heterozygotes.

Clearly, these two studies appear to be in conflict. In an analysis of these data, Bishop and Hoppe pointed out that precise estimates were difficult since the study of FitzGerald and his co-workers relied on a small number of mutations while that of Athma and his colleagues analyzed only a small number of breast cancers (64). Larger scale studies are required with emphasis on age of onset of breast cancer to address conclusively the potential association between mutations in ATM and risk of developing breast cancer. In a workshop held last November in Clermont-Ferrand, results were presented from studies in several countries, but the connection between AT heterozygosity and breast cancer remains unresolved.

For AT carriers, the picture that emerges is that while epidemiological studies point to a threefold to fourfold increased risk for breast cancer, there remains uncertainty as to whether this is supported by mutation analysis of the ATM gene. Screening of increased numbers of patients with breast cancer is required to support a small moderate increased relative risk for AT heterozygotes. It seems unlikely that the intermediate cellular radiosensitivity in AT carriers increases the risk of breast cancer during mammographic screening, at least when this procedure is restricted to women over the age of 40 (65). However, Appleby and his colleagues suggest that screening for ATM mutations in cancer patients may not be of value in predicting adverse reactions in the AT heterozygotes (66).

In a study by Clarke and his co-workers, it was shown that normally ATM is not upregulated after DNA damage, but in the proliferative myoepithelium of sclerosing adenosis, ATM expression increases significantly. The authors also indicated that there are factors other than ATM mutations that can dramatically influence ATM expression in the breast and that these factors should be considered for their possible implications in carcinogenesis (67).

In addition, Laake and his colleagues found three distinct regions at 11q23.1 that may be involved in breast development; one between the markers D11S1294 and D11S1818, a second at APOC-3, and a third that is possibly the ATM gene itself (68). Waha and his co-workers found that concentrations of the ATM transcripts in breast carcinomas were low, in benign lesions levels were intermediate and in normal breast tissue specimens levels were highest (69). Moreover, in the particular note of Rio and his colleagues, it was indicated that LOH of ATM was correlated with higher grade and a lower age of diagnosis in ductal breast carcinoma (70).

PTEN

Recently, two research teams separately homed in on a tumor suppressor gene, the loss or inactivation of which may be important for the progression of many cancers such as brain, breast and prostate (Table). This gene was called PTEN by the Parsons group (71) and MMCA by Steck and his colleagues and joins some 16 other known tumor suppressors (72). This gene has been mapped on human chromosome 10q23 (Fig. 1). PTEN is a

phosphatase but, unlike other phosphatases, its target is apparently not a protein. Instead, it is a fatty molecule, or lipid, that is tucked into the cell membrane. The target lipid is phosphatidylinositol-3,4,5-triphosphate (PIP3) and is a key component of one the cells major growth control pathways, acting both to stimulate cell growth and to block apoptosis, a form of cell suicide that can keep damaged cells from proliferating. By stripping away one of PIP3's three phosphates, it appears, PTEN reins in the growth pathways and allows cell suicide to proceed, keeping cell population in check.

Conversely, loss of PTEN during tumorigenesis presumably keeps the PIP3 pathway inappropriately activated, allowing the mutated cells to grow unchecked when they should die (73).

In preliminary screens, mutations of PTEN were detected in 31% (13/42) of glioblastoma cell lines and xenografts, 100% (4/4) of prostate cell lines, 6% (4/65) of breast cancer cell lines and xenografts, and 17% (3/18) of primary glioblastoma (71).

Tonks and his colleagues have reported that the normal gene inhibits the growth of the cells. However, the gene with the mutation has lost its ability to prevent the cells from proliferating. Tonks's team suggests that mutant PTEN lacks lipid phosphatase activity, but that its protein phosphatase activity remains intact (74). Webster Cavenee and Frank Furnari have also looked at a handful of PTEN mutants, and, in a test tube assay, have found that every mutation that renders the protein useless as a tumor supressor, eliminates its lipid phosphatase activity (75).

Although Wigler and the Parsons team detected PTEN in breast cancer, new studies in this area seemed to be controversial. Feilotter and his team analyzed the chromosomal region of the 10q23 and the PTEN gene in human sporadic breast cancer. They used a combination of denaturing gradient gel electrophoresis and SSCP analysis to investigate the presence of PTEN mutations in tumor with LOH in this region. They did not detect mutations of PTEN in any of these tumors. Their data show that in sporadic breast carcinoma, loss of heterozygosity of the PTEN locus is frequent, but mutation of PTEN is not. They suggested that these results are consistent with loss of another unidentified tumor suppressor in this region in sporadic breast carcinoma (76).

Another team characterized a breast cancer cell line derived from a germline BRCA1 mutations carrier and found that this cell line carried an acquired homozygous deletion of the PTEN gene (77).

Bose and his colleagues analyzed in situ invasive carcinomas to determine the status of chromosome 10q23 in primary breast carcinomas. They used microsatellite markers spanning the 10q23 region to analyze allelic loss. No LOH was seen in pure intraductal carcinomas (0/20 cases). On the other hand, LOH was observed in 40% (17/42) of invasive carcinomas (P=0.0005). Interestingly, in situ lesions found in invasive tumors displayed LOH. Allelic loss was also significantly associated with loss of the estrogen receptor (P=0.011). Thus, loss of the 10q23 is strongly associated with tumor progression (78).

In contrast, other results suggest that mutation of the PTEN /MMCA does not play a major role in breast cancer (79, 80) and that germline mutations in PTEN are an uncommon case of genetic predisposition to breast cancer within the general population.

Role of Estrogen in Breast Cancer

It has been known for some time that there is a link between estrogen and cancer. Both epidemiological and cell biology studies have indicated that it contributes to the development of the top five cancers of women—those of the breast, uterus and ovaries—which together account for an estimated 240,000 new cancer cases in the United States alone (81). In recent studies, it was indicated that there may be additional genes which contribute to breast cancer risk. Much more common are multiple susceptibility genes which have low absolute risk, but potentially high population-attributable risk. One such class of genes is the one that codes for enzymes or receptors which control the metabolism and intracellular transport of estrogen. It is assumed that, among ethnic groups, genetic differences exist which affect steroid hormone metabolism and transport. According to the polygenic model developed by Henderson and Feilgeson, there are functionally important polymorphisms in genes encoding enzymes involved in steroid hormone biosynthesis and metabolism, which lead to differences in individual susceptibility to breast cancer and may interact with exogenous hormone exposures. The genes of interest are the 17β-hydroxysteroid dehydrogenase 2 (EDH17B2) gene, the cytochrome p450c17a (CYR17)

gene, and the estrogen receptor (ER) gene (Fig. 5). The primary role of steroid receptors, such as ER, is to regulate the rate of transcription of certain genes by binding as a hormone receptor complex to specific sequences of DNA called hormone response elements (HREs). Interaction between the receptor and HREs can result in either up- or down-regulation of transcription depending upon binding and action of auxiliary factors specific to the target gene and the tissue. Polymorphisms in the ER gene may affect estrogen binding and subsequent transcription in target genes (82).

On the other hand, it was found that AIB1, a member of the SRC-1 family and a steroid receptor coactivator, has amplification in approximately 10% and high expression in 64% of primary breast cancer. AIB1 protein interacted with estrogen receptors in a ligand-dependent fashion, and transfection of AIB1 resulted in enhancement of estrogen-dependent transcription. These observations identify AIB1 as a nuclear receptor coactivator whose altered expression may contribute to the development of steroid-dependent cancers (83).

Although estrogen was supposed to act mainly as a growth factor in promoting cancers, new work suggests that products it forms in the body may also initiate mutations. Cell culture studies show, for example, that estrogen metabolites can bind to DNA and trigger damage. The same compounds also produce cancer in lab animals. And recent epidemiological studies suggest that women who have reduced amounts of the enzymes that help soak up the reactive estrogen byproducts are at higher risk of developing breast cancer (81).

Metabolic genes and their role in carcinogenesis, as well as the role of the estrogen byproducts in the developing cancer are a relatively new area of research with scant information at present. Studying mutations and polymorphisms in these and other genes involved in estrogen metabolism will further our understanding of breast cancer.
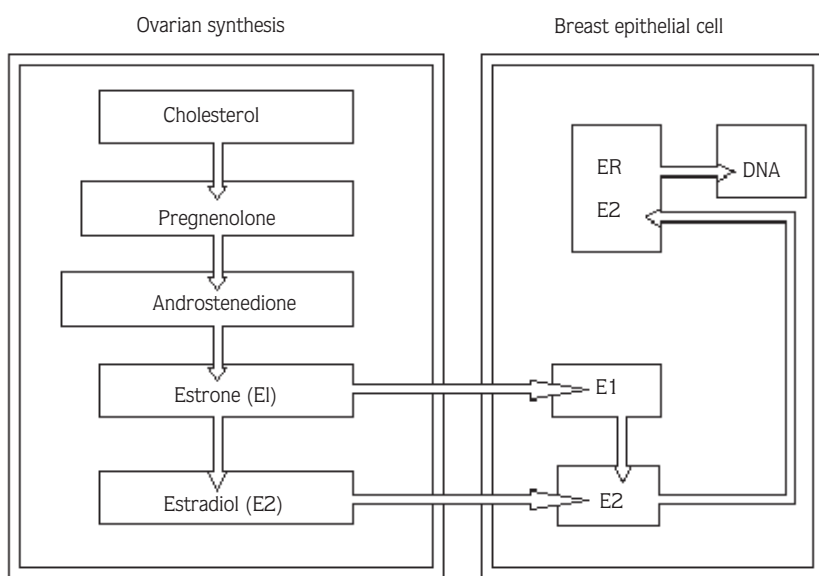

Fig. 5. Schematic presentation of CYP 17, EDH17B2 and ER in estrogen metabolism in the ovaries and breast epithelium.

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