Truncation at Conserved Terminal Regions of BRCA1 Protein Is Associated with Highly Proliferating Hereditary Breast Cancers

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Abstract

The existence of two subgroups of BRCA1-associated breast cancer (BC) families has been recently posited: the first with highly proliferating tumors, and the second composed of cases with a low proliferation rate. Our aim was to test whether the proliferation rate of BRCA1-associated breast cancers was affected by the site of the germline mutation in the BRCA1 gene. We analyzed the distribution of the mitotic index, a histoprognostic grade component shown to segregate in families, matching the PCR for germline mutation location in a series of 28 breast cancers from 20 kindreds. We observed a prevalence of highly proliferating tumors when the mutation occurs in the two terminal conserved domains of the BRCA1 protein, i.e., in the amino and carboxyl termini (P = 0.0024). Our data provide evidence for a genotype-phenotype correlation and along with their strong conservation during evolution argue for the importance of these two regions in the control of mammary cell growth.

Introduction

Germ line mutations at the BRCA1 gene are associated with the development of almost one half of hereditary BCs (1). BRCA1 encodes a ring finger-containing protein (2), putative member of the granin family (3), and is conserved in mammals (2). Its function is still undetermined. Nevertheless, evidence is mounting that BRCA1 acts as a tumor suppressor gene (4), and is involved in cell proliferation and differentiation processes of mammary epithelial cells in response to hormonal stimulation (5, 6). Accordingly, recent data show that BRCA1-associated BCPs are highly proliferating tumors (7), and that the MI, a morphological parameter widely used to evaluate tumor proliferation, segregates as a genetic trait in families (8).

More than 100 different BRCA1 germ line mutations have been identified, and the majority (90%) correspond to frameshift, nonsense, or splice mutations predicted to lead to a truncated BRCA1 protein (9, 10). Mutations are dispersed throughout the coding sequence, and only a few of them appear to be relatively common (9). However, BRCA1-associated BC families have variable phenotypic manifestations potentially in relationship to distinct molecular defects (1).

instance, mutations predicted to lead to a truncated protein before exon 13 (with a change point between codons 1435 and 1443) seem to be associated with a higher incidence of ovarian cancer in families (11). Another genotype-phenotype correlation was anticipated by the description of two subgroups of BRCA1-associated BC families (8). The first subgroup (78%) was composed of cases with high proliferation rates, and the second subgroup (22%) was composed of cases with low proliferating BCs. To test for the existence of two types of BRCA1 alleles differently affecting breast tumor growth, we compared the site of the mutation in the BRCA1 gene and the score of proliferation (MI) in a series of BC cases from families for which a BRCA1 germ line mutation has been identified.

Patients and Methods

Family and Patient Selection. Families with a strong history of breast and ovarian cancers were identified from the records of the French Cooperative Network (12) and from our cancer genetics clinics. Blood samples were collected from 152 families who had either three or more female first- or second-degree relatives affected with ovarian and/or breast cancer or two BC cases (one of which was diagnosed by age 40 years or two cases by age 45 years). Molecular studies were performed, and pathological material was collected in the meantime. Germ line mutations were found in 41 families. Since missense mutations are rare events in the BRCA1 gene, and up to now of unproven biological significance, except those which take place in the ring finger domain (9, 10), they were excluded from the study. Twenty families (49%) were selected on the basis of both the identification of a BRCA1 germline mutation that leads to a truncated protein and the availability of paraffin blocks from at least one woman who is a gene carrier with BC (Table 1). To avoid an obvious bias of sampling, the mutation sites were compared between our series of 20 families and those reported by an international group of investigators (9). We did not observe a significant difference (P = 0.19).

In addition, we have also compared the mutation spectrum of the 20 selected families with those for which blocks were not available. No significant difference was observed (P = 0.24). The selected families were all Caucasian. Nine of them were breast/ovarian cancer families. Blocks from 28 BC cases were collected. All but one were primary tumors, the remaining corresponded to a lymph node metastasis.

Mutation Analyses of BRCA1. To identify BRCA1 germ line mutation, direct DNA automated fluorescent sequencing analyses were conducted. Both DNA strands were sequenced. Briefly, PCR was performed using 40 pairs of primers (19 pairs for exon 11 and 1 pair for the remaining exons), as described previously (8), in a 25-μl volume containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 12 μM deoxynucleotide triphosphates, 50 μM of each primer, and 25 ng of genomic DNA. The reactions were carried out using a Perkin Elmer/Cetus thermal cycler model 9600. The PCR products were then purified with the Gene Clean Kit (BIO 101 Inc., La Jolla, CA) and resuspended in 25 μl dH₂O. Ten μl of purified fragments were used for sequencing with an AmpliTaq Dye Terminator Cycle Sequencing kit, using the PCR primers mentioned above, and the reactions were analyzed on an ABI 373A automated DNA sequence. In nine families (F15, F49, F73, F75, F104, F233, F342, F417, and F461) and in eight families (F358, F475, F548, F735, F779, F1199, F1348,
and F1381), prior to direct DNA sequencing, denaturing gradient gel electrophoresis and single-strand conformation polymorphism were performed, respectively.

Pathological Analysis. Diagnoses were reviewed independently by two pathologists from different institutions according to the histological typing of BC. In case of discrepancy, results were discussed between the two pathologists, and a consensus was reached. The MI from the Scarff-Bloom-Richardson histoprotostatic grade was scored according to the recommendations of Contesso et al. (13): no or one mitosis, 1; two mitoses, 2; and three or more mitoses, 3, per high-power field (×400).

Statistical Methods. χ^2 and Fisher exact tests were used to compare each parameter. When an expected cell value was less than five, the Fisher exact test was used. Statistical analyses were performed using the EPI-INFO version 5.01 package.

For the contingency table (R×C) with sparse data, as for the MI repartition, the exact P value for the Kruskal-Wallis test (14, 15) was computed using the StatXact package (Cytel Software Corporation).

Results and Discussion

In 20 families where the mutation was predicted to result in a truncated protein, a BC paraffin block from at least one gene carrier was available (Table 1). BRCA1 mutation analysis was performed on genomic DNA, with or without denaturing gradient gel electrophoresis or single-strand conformation polymorphism, to screen for DNA variants prior to direct sequence analysis. Fourteen distinct mutations were identified (Table 1): one in exon 2 corresponded to the recurrent 185delAG mutation; one in each of exons 3, 5, 10, and 23; seven alterations in exon 11, of which the recurrent 4184del4 mutation was seen twice; and seven mutations in exon 20, six of which corresponded to the recurrent 5382insC.

The MI is a morphological parameter which estimates tumor proliferation (16). It is a constitutive component of the histoprotostatic grade (13), a determinant parameter widely used to evaluate the aggressiveness of numerous tumors, and thus to select for the initial therapeutic strategy. Recently, we have proposed that the grade, through its MI component, could be the morphological translation of the loss of tumor suppressor gene function of BRCA1 and a consequence of the germ line mutation (8). Therefore, 28 BCs were collected and scored for the MI (Table 1). Among them, six tumors with a low proliferation rate (MI-1), six tumors with an intermediate proliferation rate (MI-2), and 16 with a high proliferation rate (MI-3) were found (Table 1). The highly proliferative tumors were mainly associated with a mutation near the 5' and 3' ends of BRCA1 (Fig. 1).

In contrast, the six tumors with a low proliferation rate were associated with gene mutations in exons 10 and 11. This striking distribution suggests that mutations might not be functionally equivalent. The phenotypical expression of BC, in terms of proliferation and prognosis, may be influenced by the location of the mutation in the BRCA1 gene.

The murine Brca1 gene has recently been characterized (17, 18). The mouse primary amino acid sequence shows a relatively low level of identity (60%) with the human BRCA1 protein, but two stretches with a higher degree of conservation have been identified (17, 18). The first contains the so-called ring finger domain near the amino terminus, and the second is defined by 160 amino acids near the carboxyl end of the BRCA1 protein, distinct to the granin consensus motif (Ref. 3; Fig. 1). For other tumor suppressor proteins, highly conserved regions in both humans and mice have been instrumental in pointing out domains of particular biological relevance in which deleterious mutations tend to cluster (19). Therefore, we examined the possibility that the phenotypic expression of BRCA1 mutations may be related to the degree of interspecies conservation of the region in which they occur. A significant difference in the pattern of proliferation and the location was observed between conserved and variable regions (Table 2). In conserved regions, 80% of the tumors were associated with MI-3, 20% with MI-2, and 0% with MI-1 versus only 31% with MI-3, 23% with MI-2, and 46% with MI-1 in the variable regions (P = 0.0024). This result suggests that terminal conserved regions are prominent in the control of cell proliferation. Thus, one can define in the BRCA1 protein two regions in which truncation results in a high proliferation rate in alternance with one associated with a lower proliferation rate. The existence of tumors with MI-3, for which the mutation lies in the variable regions, could be explained by the occurrence of additional somatic genetic changes at the time of diagnosis (Fig. 1).

A similar genotype-phenotype correlation with alternance of the phenotypic expression has been observed for mutations, leading to a truncation of the APC protein in familial adenomatous polyposis. It has been shown that congenital hypertrophy of the retinal pigment epithelium is present in patients when the mutation occurs between codons 413 and 1387, in contrast to patients with mutations between codons 136 and 302 and between 1445 and 1578 (20, 21). Furthermore, attenuated forms of familial polyposis (AAPP) are associated with truncation between codons 538 and 902 and between 1387 and 1601.
with mutations in exons 3 and 4 near the 5' end (22), whereas deletion at codon 1309 in exon 15 is associated with a severe form (23).

Our finding stresses the interest of interspecies analyses by pointing out conserved regions that are potentially critical to the protein function. The two conserved terminal domains of the BRCA1 protein seem to be both determinants in cell proliferation control. It may be speculated that the potential binding ring finger motif and the second conserved domain are involved in regulating the expression of genes in response to hormonal stimulation and sites important in oligomerization or in protein-protein interaction. Therefore, disruption of the protein in these regions will compromise dramatically its capability to control the cell growth and will lead to BC development. The mechanism underlying the prevalence of low proliferative BCs associated with truncation in variable regions of BRCA1 protein remains elusive. A possible explanation could be the production of mutant proteins which partially inhibits cell growth in vivo, but not tumor formation, either by alternative splicing, BRCA1 displaying a considerable heterogeneity of splice variants (2) and as suggested in AAPC (24), or in unmasking functional domains in the truncated peptides. Accordingly, a BC homozygote for a nonsense mutation in exon 11 has recently been reported (25). This is quite unexpected for a high penetration gene for which homozygosity is usually considered as lethal. Further experimental approaches are needed to test for the biological relevance of the different domains and the effects of the BRCA1 mutant proteins affecting these regions in BC development. In addition, analyses of ovarian cancer proliferation should be conducted. Differences may exist between breast and ovarian cancers, as demonstrated by the transfection assay in sporicid cancer cell lines (6). Alternatively, it is possible that the genetically primed tumors differ as a whole from the sporadic cases.

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### References


Survivin, a member of the inhibitor of apoptosis protein (IAP) family. We investigated the antiapoptotic mechanism of Survivin, as well as its expression in human tumor cell lines. Survivin binds specifically to the terminal amino acid motif termed BIR, which is present in one to three copies. The Survivin protein is abundantly expressed during fetal development in humans, but rarely present in adult tissues. Overexpression of Survivin in a lymphokine-dependent hematopoietic cell line inhibits certain caspase-family cell death proteases, implying that Survivin functions as an inhibitor of cell death proteases, caspase-3 and -7, but not to the proximal initiators of apoptosis (17, 18). Survivin protects cells from apoptosis induced by death stimuli such as TNF-α, Fas, or etoposide. Although Survivin, which is commonly expressed in human tumor cell lines, can bind to and inhibit various caspases, the antiproteolytic activity of Survivin is more potent on the distal than the proximal portions of the protease cascades, functioning as an effector rather than a initiator of apoptosis. Survivin is overexpressed in certain human tumors, including lung, breast, and prostate carcinomas, with the lowest levels in renal cancers. These findings indicate that the Survivin IAP family proteins may play an important role in resistance to chemotherapy and radiation (1).
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