

RAPID COMMUNICATION

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The murine homolog of the human breast and ovarian cancer susceptibility gene *Brca1* maps to mouse chromosome 11D

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Abstract The recently cloned human breast and ovarian cancer susceptibility gene, *BRCA1*, is located on human chromosome 17q21. We have isolated murine genomic clones containing *Brca1* as a first step in generating a mouse model for the loss of *BRCA1* function. A mouse genomic library was screened using probes corresponding to exon 11 of the human *BRCA1* gene. Two overlapping mouse clones were identified that hybridized to human *BRCA1* exons 9–12. Sequence analysis of 1.4 kb of the region of these clones corresponding to part of human exon 11 revealed 72% nucleic acid identity but only 50% amino acid identity with the human gene. The longest of the mouse *Brca1* genomic clones maps to chromosome 11D, as determined by two-color fluorescence in situ hybridization. The synteny to human chromosome 17 was confirmed by cohybridization with the mouse probe for the NF1-gene. This comparative study confirms that the relative location of the *BRCA1* gene has been conserved between mice and humans.

Introduction

Breast cancer is the second leading cause of cancer deaths among women in industrialized countries (Marshall 1993). Of all breast cancer cases, 10% have a strong genetic component. Germline mutations in the breast and ovarian cancer susceptibility gene, *BRCA1*, appear to ac-

count for about 45% of all inherited breast cancer cases and for 80% of inherited breast and ovarian cancer cases (Easton et al. 1993). The human *BRCA1* gene has recently been identified using positional cloning methods (Miki et al. 1994; Futreal et al. 1994) and maps to human chromosome 17q21. It encodes a protein that contains a ring finger domain in its amino-terminal region. The gene is broadly expressed in human (Miki et al. 1994) and mouse (Marquis et al. 1995), although the function of the protein is still unknown. *BRCA1* mutations associated with breast cancer are found throughout the coding region and result in a truncated or absent protein in more than 70% of the cases (Shattuck-Eidens et al. 1995). Tumors from patients with *BRCA1* mutations display loss of heterozygosity of 17q21, suggesting that *BRCA1* is a tumor suppressor gene and that the mutant alleles are probably null alleles or loss of function mutations.

The function of the *BRCA1* gene, its role in normal development, in tumor formation, and its interaction with other genes will be difficult to study in humans. In addition, early consequences of impaired function of tumor suppressor genes are difficult to pursue in human carcinomas. This applies in particular to cancers in which early lesions are not readily diagnosed, e.g., breast carcinomas. Transgenic animals that carry null mutations of candidate tumor suppressor genes are well-suited experimental tools for a molecular dissection of tumor suppressor gene functions and their effects on genomic instability. As a step toward a mouse model for *Brca1* mutations, we report here the isolation of the mouse homolog of the human *Brca1*-gene, the partial sequence analysis, and the mapping of the gene to mouse chromosome 11D using two-color fluorescence in situ hybridization (FISH).

Materials and methods

A mouse 129Sv genomic library (Stratagene) was screened using probes corresponding to nucleotides 2263–4215 of human *BRCA1* exon 11. Two overlapping murine *BRCA1* clones were isolated and analyzed by restriction mapping and Southern blot hybridization using probes corresponding to human exons of *BRCA1*.

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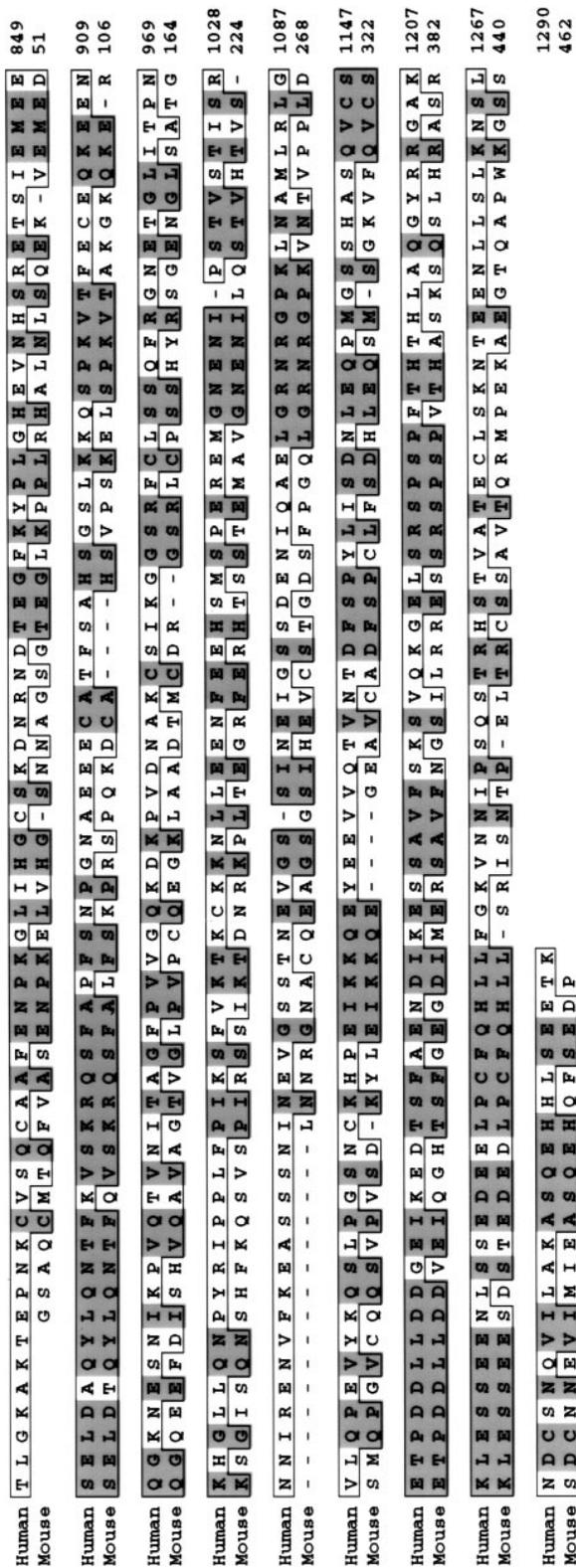


Fig. 1 Alignment of the partial mouse *Brcal* protein sequence (derived from the 1.4-kb *Bam*H1 subclone) to the human *BRCA1* gene (amino acids 790–1290). Identical residues are shaded. Sequence similarities are boxed. The IUPAC single letter amino acid code is used. Amino acids are numbered according to the GenBank entries of the human and mouse sequences, accession nos. U14680 and U33835, respectively

Portions of the mouse *Brcal* genomic clones were subcloned into pBluescriptIIKS(+) (Stratagene) and sequenced using manual (fmole kit, Promega) or automated (Applied Biosystems) methodologies as recommended by the relevant manufacturer. Sequence analysis was performed using the Edit Seq and Seqman components of the DNASTar package. Sequence alignment was performed using the Clustal algorithm component of the Megalign program (DNASTar). Amino acid similarity was defined as “ ≤ 2 distance units” utilizing the PAM250 scoring matrix. All DNA and protein sequences have been deposited in the GenBank, accession no. U33835.

Primer sequences were chosen with the PrimerSelect program (DNASTar) and oligonucleotides were synthesized with an Expedite (Millipore) DNA synthesizer. Primer information and polymerase chain reaction conditions have been deposited in the Mouse Genome Database.

The chromosomal location of the clones containing the mouse *BRCA1* gene and the co-localization with the mouse *NF1* clone were determined using two-color FISH. Metaphase chromosomes were prepared from lymphocytes obtained after splenectomy of a B6C3 mouse (C57BL/6 \times C3H)F₁. The spleen was crushed using a mouse spleen homogenizer and the cells were cultured for 48 h in RPMI medium 1640 containing 20% fetal bovine serum, 6 μ g/ml concanavalin A, and 86.4 μ M β -mercaptoethanol. Cells were synchronized with methotrexate (17 h, 4.5 μ g/ml) followed by incubation with 5-bromo-2'-deoxyuridine (30 μ g/ml) and 5-fluoro-2'-deoxyuridine (0.15 μ g/ml) for 5 h to release the S-phase block. Colcemid (0.1 μ g/ml) was added for 10 min. Hypotonic treatment using KCl (0.55%) and fixation by methanol/acetic acid (3:1) were performed following standard procedures (Barch 1991). Prior to FISH, the metaphase preparations were pretreated with RNase A (0.1 mg/ml, for 1 h at 37°C) and pepsin (0.1 mg/ml, for 10 min at 37°C) followed by formalin fixation (1%, for 10 min at room temperature). The *BRCA1* genomic clone was labeled with biotin-16-dUTP (Boehringer Mannheim, Indianapolis, Ind.), and the probe of the *NF1* gene was haptenized with digoxigenin-11-dUTP (Boehringer Mannheim) using standard nick-translation reactions.

Hybridization, detection, image acquisition, and pseudocoloring were performed as described by Ried et al. (1992) with the following modifications. Each probe (300 ng) was used for precipitation in the presence of 10 μ g mouse Cot-1 DNA. The biotinylated sequences were visualized with fluorescein isothiocyanate (FITC) conjugated to avidin (Vector, Calif.). The digoxigenin-labeled sequences were detected by indirect immunofluorescence with mouse anti-digoxin and rabbit anti-mouse Ig-tetramethylrhodamine isothiocyanate (TRITC) antibodies (Sigma, St. Louis, Mo.). Hoechst and 4'-6-diamidino-2-phenylindole dihydrochloride staining were performed to visualize the G-like banding pattern. Images were acquired using a cooled charge coupled device camera (Photometrics, Tucson, AZ) connected to a Leica DMRBE epifluorescence microscope.

Results and discussion

As part of an ongoing effort to study the function of the breast and ovarian cancer susceptibility gene, *BRCA1*, in mouse, we isolated genomic clones from a mouse library using human sequences (exon 11) as a probe. Two genomic clones (13 kb and 18 kb) were analyzed using restriction mapping, Southern blot analysis, and DNA-sequencing. The larger clone contained sequences corresponding to human exons 9–12 (data not shown). A *Bam*H1 1.4-kb fragment corresponding to part of the large (3.4 kb) human exon 11 was subcloned and sequenced. When used as a probe against mouse genomic DNA, this fragment yielded a pattern consistent with a single copy gene (data not shown). This *Bam*H1 fragment revealed a

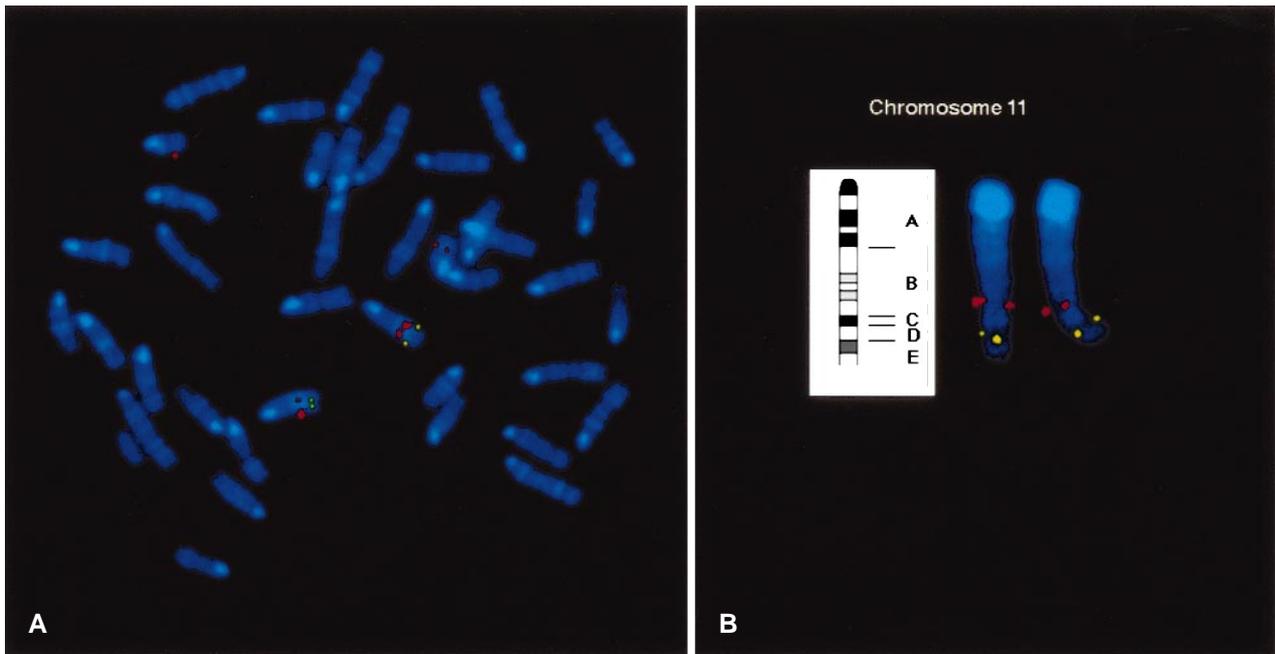


Fig. 2 **A** Two-color FISH using genomic clones for the murine *Brcal* (FITC, green fluorescence) and NF-1 genes (TRITC, red fluorescence) on mouse chromosomes. Note that both clones are located on chromosome 11. The NF1 clone shows crosshybridization to chromosome 19C3-D1. **B** Composite of two representative examples of mouse chromosome 11 after two-color FISH and the respective idiogram. The murine *BRCA1* genomic clone maps to chromosome 11D (green signal). Chromosome identification was facilitated by colocalization of the mouse homolog of the NF1 gene to 11B4-5 (red signal)

DNA homology of 72% compared with the sequence of the human *BRCA1* gene and contained a single large open reading frame.

Even though the sequence presented here only represents 25% of the mouse *Brcal* coding region (assuming it is the same size as the human protein, viz., 1863 amino acids), a comparison of the two sequences may shed light on the importance of certain residues. The open reading frame of the *Bam*H1 subclone roughly corresponds to residues 795–1290 of the human protein. Surprisingly, the amino acid sequence of the murine open reading frame compared with the human amino acid sequence reveals a conservation of only 50% (Fig. 1). Sequence divergence of this degree is observed in approximately 20% of all mouse/human homologous pairs currently in the Genbank (W. Makalowski, personal communication). When compared with the human sequence, the mouse protein has 10 deletions, ranging from a single residue to one large interval of 16 amino acids. Only two residues appear as insertions in the mouse sequence compared with the human (Fig. 1).

We have further analyzed the sequence at the codon level. When using the method of Li et al. (1985) to correct for “back-mutations”, differences between the mouse and human genes are observed with an equal frequency at all three codon positions, 1st position: $K1 = 0.38$, 2nd: $K2 =$

0.33 , 3rd $K3 = 0.36$, where K_n is the fraction of changes at each position. The ratio of synonymous (changes that do not result in an altered amino acid) to non-synonymous changes is 1.25. This is considerably less than the average ratio (synonymous:non-synonymous = 7.1) observed by Wolfe and Sharp (1993) when comparing 363 rat and mouse homologous pairs. Taken together, these results imply that, at least for the interval sequenced, the protein sequence of *Brcal* has been under relaxed selection during the 80 million years since mice and humans diverged from a common ancestor.

Interestingly, when mutations found in humans in this interval are considered, the majority (> 80%) of those associated with breast cancer lead to protein truncation (frameshifts, stop codons) (Shattuck-Eidens et al. 1995). Only five human missense mutations have been reported in the portion of the human gene shown in Fig. 1. Of these, four are population polymorphisms. Two of these, viz., P871L (by convention, mutations are represented by the amino acid change and the residue number; thus, P871L indicates that the proline codon for residue 871 has been mutated to a leucine codon) and K1183R, have equivalent residues in the mouse. In each case, the mouse clone contains the same residue as one of the human polymorphisms, viz., leucine and arginine, respectively. Two additional human polymorphisms fall into the 16 amino acids deleted in the mouse. The single reported pathologic missense mutation in this interval is T826K, which is conserved as a threonine in mouse. The ability of the *BRCA1* protein to tolerate amino acid substitutions may account for the high percentage of protein-truncating mutations when disease alleles are analyzed.

FISH analysis with one of the genomic clones containing the murine *Brcal* gene was performed on mouse metaphase chromosomes. Signals were observed in 85% of 15 randomly selected metaphase spreads. No additional

chromosomal localization was found for the *Brcal*-specific signals. The results show that the mouse *Brcal* maps to chromosome 11D (Fig. 2), which is syntenic to human 17q21. We have confirmed that this hybridization signal is located on mouse chromosome 11 by co-hybridization of a differentially labeled genomic clone for the tumor suppressor gene neurofibromatosis 1 (NF1). The NF1 gene maps to human chromosome 17q11.2 (Faine et al. 1989) and to mouse chromosome 11 (Bernards et al. 1994). In addition, the mouse NF1 genomic clone shows cross-hybridization to chromosome 19. Mouse chromosome 11 and human 17q contain one of the largest contiguous regions of conservation between the two species. Over 40 genes have been mapped to the equivalent interval (Lossie et al. 1994). The presence of the mouse *Brcal* gene in this interval is consistent with these data.

We have cloned and mapped the mouse homolog for *Brcal*. The reagents generated here should be useful for further analysis of BRCA1 expression and function in mammalian development and tumorigenesis.

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