Oncogene (2001) 20, 2544–2550

www.nature.com/onc

A targeted mouse *Brca1* mutation removing the last BRCT repeat results in apoptosis and embryonic lethality at the headfold stage

Peter Hohenstein[1,2], Menno F Kielman[1], Cor Breukel[1], L Michelle Bennett[3,5], Roger Wiseman[3], Paul Krimpenfort[4], Cees Cornelisse[2], Gert-Jan van Ommen[1], Peter Devilee[1,2] and Riccardo Fodde*[,1]

[1]*Department of Human & Clinical Genetics, Leiden University Medical Center, P.O.Box 9503, 2300 RA, The Netherlands;* [2]*Department of Pathology, Leiden University Medical Center, P.O.Box 9600, 2300 RC, The Netherlands;* [3]*National Institute of Environmental Health Sciences, Rm C428, building 101, 111 Alexander Drive, Research Triangle Park, NC 27709, USA;* [4]*Division of Molecular Genetics, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands*

A mouse model with a targeted mutation in the 3′ end of the endogenous *Brca1* gene, *Brca1*^{1700T}, was generated to compare the phenotypic consequences of truncated *Brca1* proteins with other mutant *Brca1* models reported in the literature to date. Mice heterozygous for the *Brca1*^{1700T} mutation do not show any predisposition to tumorigenesis. Treatment of these mice with ionizing radiation or breeding with *Apc*, *Msh-2* or *Tp53* mutant mouse models did not show any change in the tumor phenotype. Like other *Brca1* mouse models, the *Brca1*^{1700T} mutation is embryonic lethal in homozygous state. However, homozygous *Brca1*^{1700T} embryos reach the headfold stage but are delayed in their development and fail to turn. Thus, in contrast to *Brca1*^{null} models, the mutant embryos do not undergo growth arrest leading to a developmental block at 6.5 dpc, but continue to proliferate and differentiate until 9.5 dpc. Homozygous embryos die between 9.5–10.5 dpc due to massive apoptosis throughout the embryo. These results indicate that a C-terminal truncating *Brca1* mutation removing the last BRCT repeat has a different effect on normal cell function than does the complete absence of Brca1. *Oncogene* (2001) **20,** 2544–2550.

Keywords: *Brca1*^{1700T}; mouse model; hereditary breast cancer; p53; genotype-phenotype correlation; DNA damage

Introduction

Mutations in the BRCA1 tumor suppressor gene confer a strongly elevated risk to develop breast and ovarian cancer. Several studies have shed light on the function of the product of the *BRCA1* gene. A variety of proteins have been shown to interact with BRCA1 directly or indirectly, although the physiological relevance of some of these interactions remains to be elucidated. However, two recurring themes are evident. BRCA1 appears to have a function in the maintenance of genomic integrity. BRCA1 co-localizes with RAD51, a protein involved in homologous recombination during meiosis and repair of dsDNA damage (Scully *et al*., 1997a,b). This and the direct interaction with the hRad50-hMre11-p95 complex, involved in non-homologues end joining (NHEJ) of dsDNA breaks, recombinational repair, checkpoint control, and in the formation and processing of meiotic DSBs (Zhong *et al*., 1999), point towards a function for BRCA1 in the repair of dsDNA breaks and maintenance of genomic integrity. Accordingly, murine cells devoid of normal Brca1 function have been found to be sensitive to γ-radiation (Shen *et al*., 1998) and show defects in homology-directed repair (Moynahan *et al*., 1999). Moreover, other data suggest a role for BRCA1 in transcription-coupled repair (Gowen *et al*., 1998). On the other hand, BRCA1 can function as a transcriptional co-activator (Chapman and Verma, 1996; Monteiro *et al*., 1996), apparently specific for p53 (Ouchi *et al*., 1998; Zhang *et al*., 1998). The recent identification of a large protein complex encompassing BRCA1 and several other proteins involved in the detection of and response to DNA damage suggests that BRCA1 may coordinate the response to different types of damage (Wang *et al*., 2000).

Since the identification of *BRCA1* (Miki *et al*., 1994), over 800 different mutations have been described (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/). These are spread throughout the gene and include missense and truncating mutations or genomic deletions. Although stable expression of the mutant peptides has not been shown, different genotype-phenotype correlations have been described (Gayther *et al*., 1995; Grade *et al*., 1997; Sobol *et al*., 1996, 1997). The latter suggests that these proteins are stably expressed and may have a modulating effect on tumorigenesis. This also indicates that the mechanisms underlying *BRCA1*-mediated early-onset breast and ovarian cancer may differ among tumors with specific *BRCA1* mutations.

*Correspondence: R Fodde
[5]Current address: Lawrence Livermore National Laboratory, BBRP/L-448, P.O. Box 808, 7000 East Avenue, Livermore Ca, 94551, USA
Received 14 November 2000; revised 6 February 2001; accepted 12 February 2001

A useful method to study the effect of *BRCA1* mutations on physiological processes is the generation and analysis of mice with targeted mutations in the endogenous *Brca1* gene. However, the *Brca1* mutant mice described to date are either *null* mutants lacking any *Brca1* derived protein, or interfere with the alternative splicing of exon 11 (Gowen *et al*., 1996; Hakem *et al*., 1996; Liu *et al*., 1996; Ludwig *et al*., 1997; Shen *et al*., 1998; Xu *et al*., 1999b). To study the effect of different BRCA1 mutations on normal cell function and predisposition to tumorigenesis additional mouse models are needed. Here, we report the generation and molecular and phenotypic analysis of *Brca1*^{1700T}, a mouse model carrying a truncating mutation in the 3′ end of the endogenous *Brca1* gene. This mutation removes the last BRCT repeat and interferes with the p53-specific co-activating domain, similar to previously reported human *BRCA1* germline mutations (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/).

Results

The *Brca1*^{1700T} mouse model was generated by inserting a *neomycin* resistance gene in exon 20 of the murine *Brca1* gene (Figure 1a). The *neomycin* cassette was inserted in the same transcriptional orientation as *Brca1* to avoid anti-sense transcriptional interference as previously reported for *Apc* (Smits *et al*., 1999). The introduction of this mutation predicts the expression of a 1700 amino acids Brca1 protein lacking the last BRCT repeat (Figure 1b) (Bork *et al*., 1997; Koonin *et al*., 1996). This affects both exon 11-containing and exon 11-deficient splice variants. It has been shown that a similar truncated protein, when overexpressed *in vitro*, has a dominant-negative effect on the ability of co-transfected wild type BRCA1 to function as a co-activator of p53 (Zhang *et al*., 1998). Using conventional targeting of embryonic stem (ES) cells followed by blastocyst injection, we generated chimeric mice and heterozygous mutant mice. Since antibodies recognizing murine Brca1 are not available, we tested the expression of the mutant allele at the mRNA level. RT–PCR analysis showed that the mutant transcript is expressed at similar levels as the wild type messenger (Figure 1d).

To test the viability of homozygous *Brca1*^{1700T} mice, we intercrossed heterozygous mice, but did not find any homozygous offspring out of more than 300 animals tested, indicating that homozygosity for this mutation is not compatible with post-natal life. We therefore analysed litters from heterozygous intercrosses to study the developmental stage at which embryonic lethality occurs (Figure 2). At E8.5, homozygous mutant embryos are already severely growth-retarded. The amniotic cavity and allantois are present but the embryonic component of the homozygous embryos appears small when compared to the extra-embryonic part (Figure 2a–c). At E9.5, the mutant embryos have continued to develop and headfold and heart structures

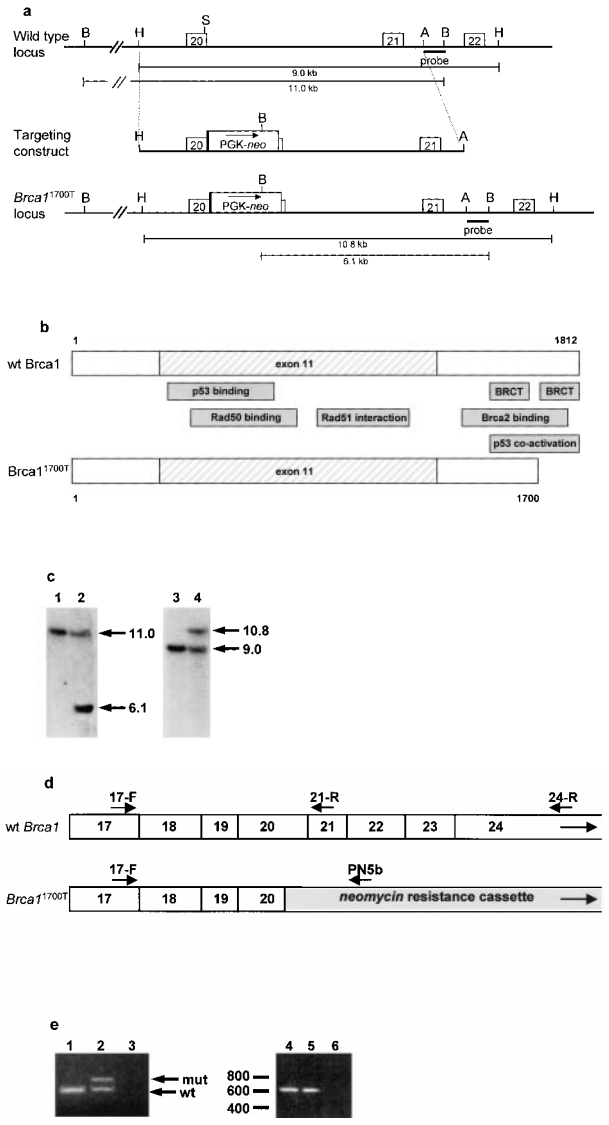

Figure 1 (**a**) Wild type *Brca1* locus and *Brca1*^{1700T} targeting construct. Abbreviations: B: A: *Apa*I, *Bam*HI, H: *Hind*III, S: *Sma*I. Figure is not drawn to scale. (**b**) Schematic representation of wild type and putative 1700T Brca1 proteins. Only relevant functional domains are depicted. The part encoded by exon 11 is shaded to emphasize that the *Brca1*^{1700T} mutation affects both exon 11-deficient and exon 11-containing splice variants. (**c**) Southern blot confirmation of correct targeting. Lanes 1 and 2: *Bam*HI digests; lanes 3 and 4: *Hind*III digests; lanes 1 and 3: *Brca1*$^{+/+}$; lanes 2 and 4: *Brca1*$^{+/1700T}$. Probe is shown in (**a**). Additional internal probes and restriction enzymes were tested and found to give the predicted fragments (not shown). (**d**) Schematic representation of wild type and predicted 1700T transcripts. Primers 17-F, 21-R and PN5b were used in a 3 primer PCR to compare expression of wild type and mutant transcripts. Primers 17-F and 24-R were used to amplify the 3′ end of *Brca1* to detect splicing artifacts as a result of the insertion of the *neomycin* resistance cassette in exon 20. (**e**) Expression analysis of the *Brca1*^{1700T} allele by RT–PCR. Amplification products are from wild type and heterozygous ES cell lines. See (**d**) for experimental design and primers. Lanes 1–3: RT–PCR products obtained with primers 17-F, 21-R and PN5b; lanes 4–6: primers 17-F and 24-R. Lanes 1 and 4: *Brca1*$^{+/+}$; lanes 2 and 5: *Brca1*$^{+/1700T}$; lanes 3 and 6: H_2O (negative control). Results were confirmed using RNA derived from adult testis (not shown)

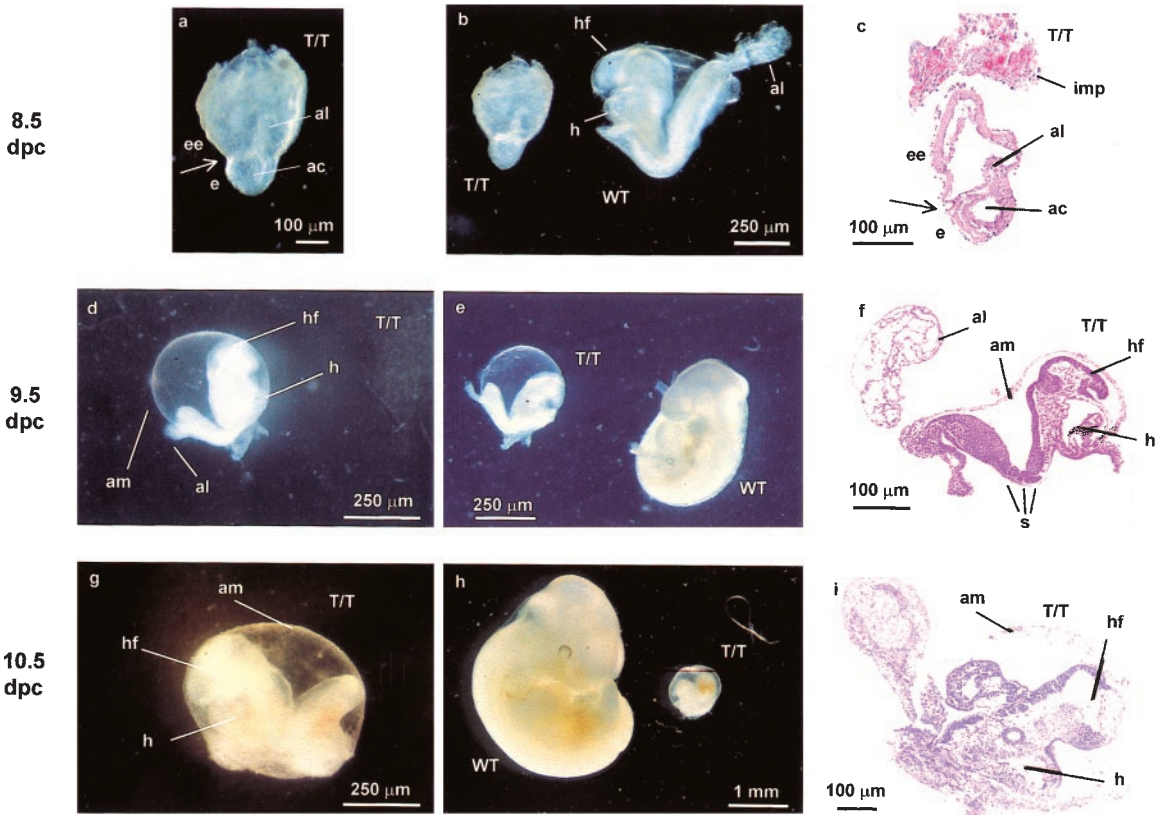

Figure 2 Analysis of homozygous *Brca1*^{1700T} embryos. (**a**–**c**) 8.5 d.p.c. (**d**–**f**) 9.5 d.p.c. (**g**–**i**) 10.5 d.p.c. Abbreviations: T/T: homozygous *Brca1*^{1700T}, wt: wild type, ac: amniotic cavity, al: allantois, am: amnion, e: embryonic component, ee: extra-embryonic component, h: heart, hf: headfolds, imp: implantation site, s: somites. Arrows indicate the boundary between embryonic and extra-embryonic components

are now clearly present. However, the tailbud of the mutant embryos is strongly enlarged while only a few somites can be observed, indicating that somitogenesis is strongly impaired (Figure 2d–f). At E10.5 *Brca1*$^{1700T/1700T}$ embryos have further increased in size, but failed to develop any further (Figure 2g–i). Microscopic sections show large numbers of apoptotic bodies. Notably, Chandler *et al*. (2001) showed that the *Brca1*^{1700T} embryonic lethality is rescued by breeding these mice with transgenic ones generated with bacterial artificial chromosomes (BAC) encompassing either the human *BRCA1* or the murine *Brca1* gene. These results confirm that the observed phenotype directly results from the targeted *Brca1*^{1700T} mutation.

The embryonic phenotype in the *Brca1*^{1700T} mouse model is clearly different from that reported for other *Brca1* knockout mouse models. Embryos homozygous for *null* mutations are extremely growth retarded at E6.5 and have died by E7.5 (Hakem *et al*., 1996; Ludwig *et al*., 1997). In these embryos p21 expression is upregulated, accompanied by a cell proliferation blockage. No apoptosis could be detected in these models, suggesting that a cell cycle arrest resulting in a proliferation block is the primary cause of embryonic lethality. In contrast, *Brca1*$^{1700T/1700T}$ cells continue to proliferate up to E9.5, as evaluated by BrdU incorporation (Figure 3a). These results were confirmed using an antibody against the Ki67 proliferation marker (not shown). At E10.5 proliferation can no longer be detected (Figure 3b). In contrast, an apoptotic response is activated between E9.5 and E10.5 as observed by using antibodies against the PARP p85 apoptotic fragment and by morphological examination of pyknotic cells (Figure 3c,d). This was confirmed using the TUNEL assay (not shown). Another feature of homozygous *null* embryos is the lack of mesoderm induction. The morphology of *Brca1*$^{1700T/1700T}$ embryos already strongly suggests that in this model mesoderm can be formed. Expression of Brachyury, a marker for early undifferentiated mesoderm, can be detected at E8.5 and E9.5. However, its expression appears to be reduced when compared with stage-matched wild type embryos (Figure 4).

Heterozygous *Brca1*^{1700T} mice have been followed for 2 years on an inbred 129Ola ($n=40$) and mixed C57BL/6JIco X 129Ola background ($n=25$), and do not show any susceptibility to tumor formation when compared to their age-matched wild type littermates ($n=38$ for 129Ola, $n=22$ for mixed background). This is in accordance with previously published results with other *Brca1* mouse mutants (Gowen *et al*., 1996; Hakem *et al*., 1996; Liu *et al*., 1996; Ludwig *et al*.,

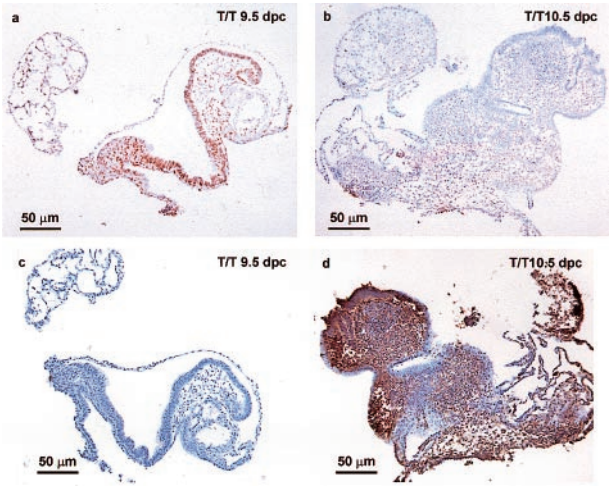

Figure 3 Analysis of proliferation and apoptosis, by BrdU incorporation and PARP p85 immunohistochemistry respectively, in homozygous Brca1^{1700T} embryos. (**a**,**b**) BrdU staining (**c**,**d**) p85 staining (**a**,**c**) 9.5 d.p.c. (**b**,**d**) 10.5 d.p.c. Results were confirmed with α Ki67 antibodies (proliferation) and TUNEL assay (apoptosis) (not shown)

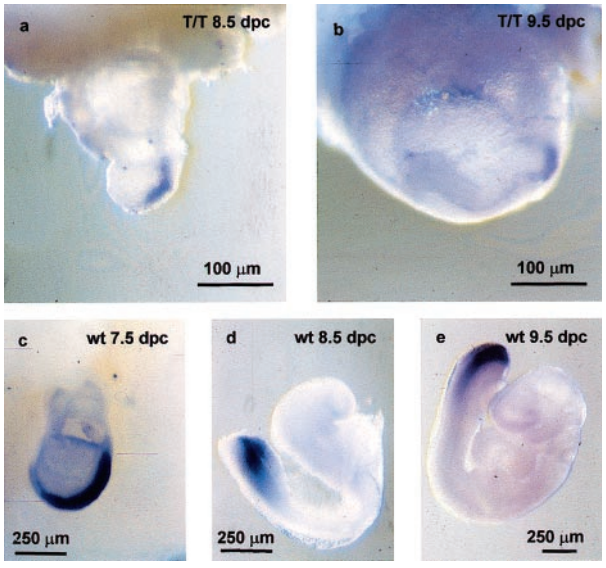

Figure 4 Whole amount RNA *in situ* hybridization analysis showing expression of Brachyury in homozygous Brca1^{1700T} embryos (**a**,**b**) and stage-matched and age-matched (littermates) wild type embryos (**c**, **d**, **e**)

1997; Shen *et al*., 1998). In view of the role played by BRCA1 in genomic integrity and repair of dsDNA breaks, we exposed 7-week-old *Brca1*$^{+/+}$ ($n=23$) and *Brca1*$^{+/1700T}$ ($n=27$) mice to γ-radiation (5 Gy full body irradiation). This treatment dramatically increases the rate of tumor formation in mammary and ovarian tissue of *Apc*^{1638N} mice (Houven van Oordt *et al*., 1997, 1999). Mice were sacrificed at 1 year of age or when moribund. We could not detect any change in the rate or distribution of tumors in *Brca1*$^{+/1700T}$ mice compared to wild type controls.

The occurrence of mammary tumors in mice with a conditional mutation removing exon 11 during lactation suggests that inactivation of the wild type *Brca1* allele is a rate-limiting event for tumorigenesis in heterozygous *Brca1* mutant mice (Xu *et al*., 1999a). As previously shown for the *Apc* gene in *Apc*$^{+/-}$ *Msh2*$^{-/-}$ mice (Smits *et al*., 2000), the frequency at which second hits occur can be increased in a mismatch repair deficient (*Msh2*$^{-/-}$) genetic background. We therefore crossed the *Brca1*^{1700T} mice with *Msh2*$^{\Delta 7N}$ mice (Smits *et al*., 2000), but again found no effect of the *Msh2* deficiency on the phenotype of *Brca1*^{1700T} heterozygous mice ($n=11$ for *Brca1*$^{+/1700T}$/*Msh2*$^{-/-}$, $n=9$ for *Brca1*$^{+/+}$/*Msh2*$^{-/-}$).

Brca1^{1700T} mice were also bred with *Apc*^{1638N} mice (Fodde *et al*., 1994; Smits *et al*., 1998) because of their low incidence of mammary tumors (Houven van Oordt *et al*., 1997, 1999). However, no differences in tumor incidence were observed by comparison of *Apc*$^{+/1638N}$/*Brca1*$^{+/+}$ ($n=13$) mice with compound *Apc*$^{+/1636N}$/*Brca1*$^{+/1700T}$ mice ($n=15$) when analysed at 1 year of age or when moribund.

Finally, we crossed *Brca1*^{1700T} mice with *Tp53* knockout mice (Jacks *et al*., 1994). *Tp53*$^{-/-}$ animals develop T-cell lymphomas at 3–5 months of age. In heterozygous mice, sarcomas but also other neoplastic lesions have been observed between 10 and 18 months. The heterozygous *Brca1*^{1700T} mutation had no additional effect on the phenotype of *Tp53*$^{-/-}$ mice ($n=8$ for *Brca1*$^{+/1700T}$/*Tp53*$^{-/-}$, $n=5$ for *Brca1*$^{+/+}$/*Tp53*$^{-/-}$). We also studied tumor incidence in compound heterozygous *Brca1*$^{+/1700T}$/*Tp53*$^{+/-}$. Since the two genes are localized 20 cM apart on mouse chromosome 11, it is essential to distinguish between the mutant alleles in the *cis* and *trans* phases. In the *Brca1*$^{+/1700T}$/*Tp53*$^{+/-}$ *cis* mice, loss of the wild type chromosome will result in a cell where only the putative *Brca1*^{1700T} protein is expressed in a p53-deficient background. Therefore, we first bred single heterozygous mice to generate *trans* compound heterozygous animals ($n=22$) which were subsequently bred to wild type C57BL/6JIco mice to select for meiotic recombinants with both mutant alleles in *cis* ($n=26$). However, we could not detect any difference in tumor incidence in the *Brca1*$^{+/1700T}$/*Tp53*$^{+/-}$ *cis* or *trans* phases at 10 months of age when compared with age-matched *Brca1*$^{+/+}$/*Tp53*$^{+/-}$ ($n=32$) mice.

Discussion

The observed differences in embryonic lethality among mouse models carrying different *Brca1* targeted mutations provide clues for the understanding of the function of BRCA1. When compared with *Brca1 null* models, homozygous Brca1^{1700T} embryos show a delay in embryonic lethality and, in contrast, continue to proliferate and differentiate until an apoptotic response is activated. Hence, specific *Brca1* mutations are associated with different molecular and cellular phenotypic consequences.

Brca1 homozygous *null* embryos are extremely growth retarded at E 6.5 and die at E 7.5 (Hakem *et al*., 1996; Ludwig *et al*., 1997). The absence of Brca1 in these embryos is likely to result in accumulation of DNA damage. Accordingly, p53 becomes activated leading to up-regulation of p21 and the accompanying cell proliferation block. Additional mouse *Brca1* targeted alleles have been generated aimed at exon 11: Liu *et al*. (1996) reported an antisense insertion of a neomycin cassette within exon 11. The corresponding homozygous embryos show a similar phenotype as the *null* mice. In two other cases, 5′ regions of exon 11 including the splice acceptor sites have been replaced with a neomycin resistance gene (Gowen *et al*., 1996; Shen *et al*., 1998). While the model reported by Shen *et al*. strongly resembles the *null* allele in terms of timing of embryonic lethality, the mouse generated by Gowen and colleagues dies between E10.5 and E13.5 due to neuroepithelial abnormalities. In a fourth model, exon 11 has been removed using Cre-Lox technology (Xu *et al*., 1999b). In the latter case, named $Brca1^{\Delta 11}$, the homozygous embryos die at E12.5–E18.5. Unfortunately, the phenotype of the latter embryos has not been reported in great details. In view of the phenotypic differences among the above exon 11 mutants, it is difficult to speculate on the molecular consequences of these mutant *Brca1* alleles. This is further complicated by the physiological alternative splicing of exon 11 (Thakur *et al*., 1997; Wilson *et al*., 1997). Hence we will limit the discussion to the comparison between $Brca1^{1700T}$ and the *null* models. It should also be noted that differences in the genetic background of the *Brca1* mutant embryos are not likely to account for phenotypic differences. The present data have been obtained with C57BL/6J X 129Ola backcrosses. The two *null* models described previously have been obtained with identical (Hakem *et al*., 1996) or similar (C57BL/6J X 129Sv, Ludwig *et al*., 1997) backcrosses and are therefore comparable.

Different models can be envisaged for the mechanisms underlying the lethality of homozygous $Brca1^{1700T}$ embryos depending on the specific Brca1 function that is affected. On one hand, repair of DNA damage might be unaffected since the domains interacting with RAD50 and RAD51 are encoded by exon 11, upstream of the targeted exon 20 region. On the other hand, DNA repair might be impaired because the C-terminal BRCT repeat, deleted in $Brca1^{1700T}$, is often found in proteins involved in response to DNA damage (Bork *et al*., 1997). In fact, from the present data we can conclude that a single BRCT repeat is not sufficient for *Brca1* function in the mouse. BRCA1 also interacts directly with BRCA2 via its C-terminus (Chen *et al*., 1998). BRCA2 function is also linked to DNA damage repair, as shown by its direct interaction with Rad51, the sensitivity to γ-irradiation of $Brca2^{-/-}$ embryos, and the chromosomal aberrations in cells only expressing a truncated Brca2 protein (Sharan *et al*., 1997; Yu *et al*., 2000). If BRCA2 function is dependent on its interaction with BRCA1, the truncation of the C-terminus in $Brca1^{1700T}$ may result in a repair defect.

Another function of the BRCA1 C-terminus likely to be impaired in the $Brca1^{1700T}$ mouse model, is the p53 co-activating activity. *In vitro* studies have shown that an analogous mutation in the human *BRCA1* cDNA results in loss of the p53 co-activating function, and may exert a dominant-negative effect (Zhang *et al*., 1998). However, it is not likely that disturbance of the p53 co-activation domain by itself can account for the lethality of $Brca1^{1700T}$ embryos. If lack of p53 co-activation was the only cause of the observed *in utero* lethality, complete p53 deficiency (in a *Brca1* wild type background) should have a phenotype of at least comparable severity. However, $Tp53^{-/-}$ mice are viable except for a small subset of the homozygous embryos (Sah *et al*., 1995). Moreover, the fact that p53 deficiency partially rescues the embryonic lethality of $Brca1^{null/null}$ mice indicates that p53 is functional in these embryos, and that not all p53 responses are Brca1-dependent (Hakem *et al*., 1997; Ludwig *et al*., 1997).

In a unifying hypothesis, repair of DNA damage is impaired in $Brca1^{1700T}$ homozygous mice due to the lack of the last BRCT repeat and/or to the disturbed interaction with Brca2, leading to accumulation of DNA damage. In normal cells p53 is activated by DNA damage and can bind to the *Brca1* exon 11-encoded binding domain to become co-activated by the C-terminus of full-length Brca1. However, since the latter domain is absent in $Brca1^{1700T}$, p53 could be sequestered by the mutant protein and thereby functionally inactivated. Hence, a mutant BRCA1 protein might have a dominant-negative effect on both the maintenance of the genomic integrity and on the response to the resulting instability. This would explain why the embryonic lethality of homozygous $Brca1^{1700T}$ resembles that of compound $Brca1^{null/null}/Tp53^{-/-}$ embryos in timing and gross morphology. The observed apoptosis in the E10.5 $Brca1^{1700T}$ embryos should then result either from a delayed (or impaired) p53 response or from a p53-independent mechanism. We note, however, that this putative dominant-negative nature of the $Brca1^{1700T}$ protein on p53 function does not suffice to trigger tumorigenesis in the presence of the wild type *Brca1* allele.

In conclusion, the embryonic lethality in homozygous $Brca1^{1700T}$ embryos and the phenotypic differences with *null* embryos suggest that mutant Brca1 proteins may have a different effect on cell function than complete absence of Brca1. In particular, since p53 status is an important prognostic factor in breast cancer (Blaszyk *et al*., 2000), the effect of truncating BRCA1 mutations on p53 function may have predictive clinical value for *BRCA1* mutation carriers.

Materials and methods

Generation of the $Brca1^{1700T}$ mouse model

A 8 kb genomic *Hind*III/*Apa*I fragment encompassing exon 20 and exon 21 was derived from a P1 clone containing the complete murine Brca1 gene (Bennett *et al*., 1995). This

fragment was employed for the generation of the targeting construct by inserting a neomycin resistance gene under control of a PGK promoter and PGK poly A signal in a unique *Sma*I site in exon 20. A HA-tag was inserted *in frame* with the Brca1 coding sequence upstream the PGK promoter. The resulting replacement-type targeting vector was used for transfection of E14 ES cells (129Ola) according to standard procedures (Fodde *et al*., 1994). Correctly targeted clones were identified by PCR (not shown) and Southern blot analysis. Targeted clones were tested for karyotype and morphology and one clone was employed for the generation of chimeric mice through blastocyst injection.

Expression of the Brca1^{1700T} allele

Since different antibodies recognizing the HA-tag could not reproducibly show expression of the mutant protein, RNA was isolated from wild type and *Brca1*$^{+/1700T}$ ES lines or from testis from adult mice and reverse transcribed using random priming. An exon 17 forward primer (17-F: TTTgCTgAAAAATACCgCCT) was used in combination with exon 21 (21-R: AATAgACCTGTAggCCCTTgAA) and PGK promoter (PN5b: CTAAAgCgCAgGCTCCAgACT) reverse primers in a 3 primer PCR to simultaneously amplify the wild type and *Brca1*^{1700T} alleles. To exclude shorter RT–PCR products generated by alternative splicing, the exon 17 forward primer was also used in combination with an exon 24 reverse primer (24-R: TTggAgTCTTgTggCTCACTA).

Mouse strains

Brca1^{1700T} mice were bred on an inbred 129Ola background. These mice were used for breeding with *Apc*$^{+/1638N}$ C57Bl/6JIco (N22) females to generate F1 compound heterozygous mice. For all other experiments mice were backcrossed to C57Bl/6JIco (B6). N4 backcross generation mice were used for the radiation experiment. Mice were irradiated with 5 Gy full body radiation at 7 weeks of age (Smart 225, Andrex; dose rate 0.1 Gy/min.). N3 *Brca1*$^{+/1700T}$ males were bred with N6 *Msh2*$^{+/\Delta 7N}$ females to generate compound heterozygous mice which were subsequently intercrossed to generate *Msh2*-deficient mice (*Brca1*$^{+/+}$ and *Brca1*$^{+/1700T}$). N5 *Brca1*$^{+/1700T}$ males were crossed with N12 *Tp53*$^{+/-}$ females to generate *trans Brca1*$^{+/1700T}$/*Tp53*$^{+/-}$ mice. These were intercrossed to generate p53-deficient mice (*Brca1*$^{+/+}$ and *Brca1*$^{+/1700T}$) or crossed to B6 wild type mice to generate *cis Brca1*$^{+/1700T}$/*Tp53*$^{+/-}$ mice. Mice used for the analysis of embryonic lethality were N5 or further backcross generations.

Analysis of embryonic lethality

Embryos were isolated from timed matings and genotyped using PCR on yolk sac or Reicherts membrane with an intron 19 forward primer (gCTggCCTggACATgAgTgTA) in combination with an intron 20 reverse primer (gTTCTgTCA-CATAAAgAgggACT) specific for the wild type allele and the PGK promoter primer (see above) for the mutant allele. Embryos were fixed o/n at 4°C in 4% PFA, dehydrated and embedded in paraffin for sectioning. Sections were stained using standard hematoxin/eosin staining. For detection of proliferation in embryos, pregnant mice were injected with BrdU (0.1 mg/g bodyweight) 1 h prior to sacrifice. Immunohistochemistry with α-BrdU antibodies (Roche Molecular Biochemicals) was performed on paraffin sections after heat antigen retrieval and denaturation. BrdU staining results were confirmed with antibodies against the Ki67 proliferation marker (Novacastra). Apoptosis was detected using antibodies recognizing the apoptotic p85 fragment of PARP (Promega) and the TUNEL assay (Roche Molecular Biochemicals) according to the supplier's protocol. Whole mount RNA *in situ* hybridizations were done as described (Wilkinson, 1992) with a probe recognizing Brachyury (Wilkinson *et al*., 1990).

Acknowledgments
The authors would like to thank Shantie Jagmohan-Changur, Ron Smits, Heleen van der Klift, and Nandy Hofland for technical assistance and critical reading of the manuscript.

References

Bennett LM, Haugen-Strano A, Cochran C, Brownlee HA, Fiedorek Jr FT and Wiseman RW. (1995). *Genomics,* **29,** 576–581.

Blaszyk H, Hartmann A, Cunningham JM, Schaid D, Wold LE, Kovach JS and Sommer SS. (2000). *Int. J. Cancer,* **89,** 32–38.

Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul S and Koonin EV. (1997). *FASEB J.,* **11,** 68–76.

Chandler J, Hohenstein P, Swing DA, Tessarollo L and Sharan SK. (2001). *Genesis,* **29,** 72–77.

Chapman M and Verma IM. (1996). *Nature,* **382,** 678–679.

Chen JJ, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Couch FJ, Weber BL, Ashley T, Livingston DM and Scully R. (1998). *Mol. Cell.,* **2,** 317–328.

Fodde R, Edelmann W, Yang K, van Leeuwen C, Carlson C, Renault B, Breukel C, Alt E, Lipkin M, Khan PM and *et al*. (1994). *Proc. Natl. Acad. Sci. USA,* **91,** 8969–8973.

Gayther SA, Warren W, Mazoyer S, Russell PA, Harrington PA, Chiano M, Seal S, Hamoudi R, Van Rensburg EJ, Dunning AM, Love R, Evans G, Easton D, Clayton D, Stratton MR and Ponder BAJ. (1995). *Nature Genet.,* **11,** 428–433.

Gowen LC, Avrutskaya AV, Latour AM, Koller BH and Leadon SA. (1998). *Science,* **281,** 1009–1012.

Gowen LC, Johnson BL, Latour AM, Sulik KK and Koller BH. (1996). *Nature Genet.,* **12,** 191–194.

Grade K, Hoffken K, Kath R, Nothnagel A, Bender E and Scherneck S. (1997). *J. Cancer Res. Clin. Oncol.,* **123,** 69–70.

Hakem R, De la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmair A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J and Mak TW. (1996). *Cell,* **85,** 1009–1023.

Hakem R, Delapompa JL, Eli A, Potter J and Mak TW. (1997). *Nat. Genet.,* **16,** 298–302.

Houven van Oordt CWvd, Smits R, Schouten TG, Houwing-Duistermaat JJ, Williamson SL, Luz A, Meera Khan P, van der Eb AJ, Breuer ML and Fodde R. (1999). *Genes Chromosomes Cancer,* **24,** 191–198.

Houven van Oordt CWvd, Smits R, Williamson SLH, Luz A, Meera Khan P, Fodde R, Eb AJvd and Breuer ML. (1997). *Carcinogenesis,* **18,** 2197–2203.

Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT and Weinberg RA. (1994). *Curr. Biol.,* **4,** 1–7.

Koonin E, Altschul S and Bork P. (1996). *Nature Genet.,* **13,** 266–268.

Liu CY, Fleskennikitin A, Li S, Zeng YY and Lee WH. (1996). *Genes Dev.,* **10,** 1835–1843.

Ludwig T, Chapman DL, Papaioannou VE and Efstratiadis A. (1997). *Genes Dev.,* **11,** 1226–1241.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barret JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A and Skolnick MH. (1994). *Science,* **266,** 66–71.

Monteiro AN, August A and Hanafusa H. (1996). *Proc. Natl. Acad. Sci. USA,* **93,** 13595–13599.

Moynahan ME, Chiu JW, Koller BH and Jasin M. (1999). *Mol. Cell,* **4,** 511–518.

Ouchi T, Monteiro ANA, August A, Aaronson SA and Hanafusa H. (1998). *Proc. Natl. Acad. Sci. USA,* **95,** 2302–2306.

Sah V, Attardi LD, Mulligan GJ, Williams BO, Bronson RT and Jacks T. (1995). *Nature Genet.,* **10,** 175–180.

Scully R, Chen JJ, Ochs RL, Keegan K, Hoekstra M, Feunteun J and Livingston DM. (1997a). *Cell,* **90,** 425–435.

Scully R, Chen JJ, Plug A, Xiao YH, Weaver D, Feunteun J, Ashley T and Livingston DM. (1997b). *Cell,* **88,** 265–275.

Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P and Bradley A. (1997). *Nature,* **386,** 804–810.

Shen S-X, Weaver Z, Xu X, Li C, Weinstein M, Chen L, Guan X-Y, Ried T and Deng C-X. (1998). *Oncogene,* **17,** 3115–3124.

Smits R, Hofland N, Edelmann W, Geugien M, Jagmohan-Changur S, Albuquerque C, Breukel C, Kucherlapati R, Kielman MF and Fodde R. (2000). *Genes Chr. Cancer,* **29,** 229–239.

Smits R, Kielman MF, Breukel C, Zurcher C, Neufeld K, Jagmohan-Changur S, Hofland N, van Dijk J, White R, Edelmann W, Kucherlapati R, Khan PM and Fodde R. (1999). *Genes Dev.,* **13,** 1309–1321.

Smits R, van der Houven van Oordt W, Luz A, Zurcher C, Jagmohan-Changur S, Breukel C, Khan PM and Fodde R. (1998). *Gastroenterology,* **114,** 275–283.

Sobol A, Stoppalyonnet D, Bressacdepaillerets B, Peyrat JP, Kerangueven F, Janin N, Noguchi T, Eisinger F, Guinebretiere JM, Jacquemier J and Birnbaum D. (1996). *Cancer Res.,* **56,** 3216–3219.

Sobol H, Stoppalyonnet D, Bressacdepaillerets B, Peyrat JP, Guinebretiere JM, Jacquemier J, Eisinger F and Birnbaum D. (1997). *Int. J. Oncol.,* **10,** 349–353.

Thakur S, Zhang HB, Peng Y, Le H, Carroll B, Ward T, Yao J, Farid LM, Couch FJ, Wilson RB and Weber BL. (1997). *Mol. Cell Biol.,* **17,** 444–452.

Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ and Qin J. (2000). *Genes Dev.,* **14,** 927–939.

Wilkinson DG. (1992). *In Situ Hybridization: A Practical Approach*. Wilkinson, DG (ed.). IRL Press: Oxford, pp 75–83.

Wilkinson DG, Bhatt S and Herrmann BG. (1990). *Nature,* **343,** 657–659.

Wilson CA, Payton MN, Elliott G, Buaas FW, Cajulis EE, Grosshans D, Ramos L, Reese DM, Slamon DJ and Calzone FJ. (1997). *Oncogene,* **14,** 1–16.

Xu XL, Wagner KU, Larson D, Weaver Z, Li CL, Ried T, Hennighausen L, Wynshawboris A and Deng CX. (1999a). *Nat. Genet.,* **22,** 37–43.

Xu XL, Weaver Z, Linke SP, Li CL, Gotay J, Wang XW, Harris CC, Ried T and Deng CX. (1999b). *Mol. Cell.,* **3,** 389–395.

Yu VP, Koehler M, Steinlein C, Schmid M, Hanakahi LA, van Gool AJ, West SC and Venkitaraman AR. (2000). *Genes Dev.,* **14,** 1400–1406.

Zhang HB, Somasundaram K, Peng Y, Tian H, Zhang HX, Bi DK, Weber BL and Eldeiry WS. (1998). *Oncogene,* **16,** 1713–1721.

Zhong Q, Chen CF, Li S, Chen YM, Wang CC, Xiao J, Chen PL, Sharp ZD and Lee WH. (1999). *Science,* **285,** 747–750.