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PRINCIPAL INVESTIGATOR: David Chen, Ph.D.

CONTRACTING ORGANIZATION: University of California
E. O. Lawrence Berkeley National Laboratory
Berkeley, California 94720

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6. AUTHOR(S):
David Chen, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California
E. O. Lawrence Berkeley National Laboratory
Berkeley, California 94720

E-MAIL:DJChen@lbl.gov

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13. ABSTRACT ***(Maximum 200 Words)***
Brca2 is a major breast cancer susceptibility gene. Recent evidence has indicated that Brca2 is important for maintaining genomic integrity because of a role in homologous recombinational repair (HRR). Brca2 is presumed to function in homologous recombination through its interactions with Rad51. Both exons 11 and 27 of Brca2 code for domains that interact with Rad51; exon 11 codes for eight BRC motifs, while exon 27 codes for a single, distinct interaction domain. Deletion of all the Rad51-interacting domains causes early embryonic lethality in mice. A less severe phenotype is seen in mice with deletions that preserve some, but not all, of the BRC motifs. These mice can survive past weaning, but are runted, infertile, and die very young from cancer. Cells derived from such mice are hypersensitive to some genotoxic agents and exhibit chromosomal instability. Here we present analysis of mice and cells with a deletion of the single Rad51-interacting region encoded by exon 27 (all the BRC motifs are preserved). This mutation is called $brca2^{lex1}$. Mice homozygous for $brca2^{lex1}$ exhibit a shorter life span compared to control littermates, possibly due to an early onset of cancer and sepsis. No other phenotype was observed in these animals; therefore, the $brca2^{lex1}$ mutation is less severe than those with larger COOH-terminal truncations that delete some of the BRC motifs. However, at the cellular level, the $brca2^{lex1}$ mutation causes reduced viability, extreme sensitivity to the DNA interstrand cross-linking agent mitomycin C, and gross chromosomal instability, much like the more severe truncations. These results confirm that the extreme COOH-terminal region encoded by exon 27, though not essential to organismal viability, is important for the function of Brca2 at the cellular level, most probably because it is required for a fully functional interaction between Brca2 and Rad51.

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Introduction

The BRCA2 protein has been shown to physically interact with the DNA repair and homologous recombination protein RAD51, both *in vitro* and *in vivo* (Chen *et al.*, 1998b; Katagiri *et al.*, 1998; Marmorstein *et al.*, 1998; Mizuta *et al.*, 1997; Sharan *et al.*, 1997; Wong *et al.*, 1997) suggesting that BRCA2 participates in a recombination complex during cell division and DNA repair processes. Shortly after exposure of normal cells to ionizing radiation, RAD51 protein relocalizes to form discrete nuclear foci, which have been proposed to represent the assembly of multiprotein recombinational repair complexes at sites of DNA damage (Chen *et al.*, 1998a; Chen *et al.*, 1999; Haaf *et al.*, 1995). In Capan-1 human cells, which lack an intact *BRCA2* gene, formation of RAD51 foci is severely impaired (Yuan *et al.*, 1999). The latter cells are also hypersensitive to ionizing radiation and to drugs that cause DNA double-strand breaks, and show reduced repair of DNA double-strand breaks after radiation exposure (Abbott *et al.*, 1998). If loss of the interactions between BRCA2 and RAD51 results in reduced capacity for homologous repair of DNA double-strand breaks or homology-dependent repair of other lesions during DNA replication, this may in turn contribute to genomic instability, and result in the eventual mutation of genes required for control of cell growth and division.

Domains that mediate interaction with RAD51 have been identified in two parts of the BRCA2 protein. An extreme carboxy-terminal domain within exon 27, comprising amino acids 3196-3232, was first shown to mediate RAD51 binding (Mizuta *et al.*, 1997; Sharan *et al.*, 1997). Soon after, it was discovered that the eight conserved "BRC repeats" encoded by the large contral exon 11 also can function individually to bind RAD51. Homozygous mutations in mouse that delete all of the identified RAD51-interacting domains of BRCA2 causes early embryonic death, apparently due to failure of cell proliferation (Ludwig *et al.*, 1997; Sharan *et al.*, 1997; Suzuki *et al.*, 1997). Cells from very early $BRCA2^{-/-}$ embryos show an exaggerated sensitivity to ionizing radiation (Sharan *et al.*, 1997). An essentially identical phenotype of early embryonic lethality and radiosensitivity has also been reported for homozygous null RAD51 mutation in mouse (Lim & Hasty, 1996). However, targeted mutations of the mouse BRCA2 gene that preserve all or some of the BRC repeats in exon 11 can support embryonic development to term and the birth of viable pups (Connor *et al.*, 1997; Friedman *et al.*, 1998; and this report).

Morimatus has reported the generation of embryonal stem (ES) cells in which both alleles of the *BRCA2* gene have been truncated by gene targeting, using two different targeting vectors (Morimatsu *et al.*, 1998). Both truncations delete exon 27, which encodes the extreme carboxy-terminal RAD51-interaction domain of the BRCA2 protein. The two targeted alleles have been designated $BRCA2^{lex1}$ and $BRCA2^{lex2}$. $BRCA2^{lex1/lex2}$ ES cells are hypersensitive to ionizing radiation but not to ultraviolet radiation. The $BRCA2^{lex1/lex2}$ ES cells were used to generate primary mouse embryonal fibroblasts (MEF). Primary $BRCA2^{lex1/lex2}$ MEF cells show an impaired growth rate and reduced cloning efficiency as compared to primary *BRCA2+/+* MEF, and undergo premature replicative senescence as determined by colony size distribution and serial passage (3T3-equivalent) analysis.

We have reported the characterization of these cells for sensitivity to mitomycin C, which produces DNA interstrand crosslinks; a type of damage thought to require repair through homologous recombination. Here we further characterize the phenotype of mice and mouse embryonic fibroblasts (MEF) with a deletion of exon 27 (designated $brca2^{lex1}$). Compared to control mice, the $brca2^{lex1}$ mice exhibit a decreased life span that may be partially due to an early onset of cancer and sepsis. The

brca2^{lex1} allele exhibits a normal Mendelian pattern of inheritance, indicating no loss of viability in homozygous embryos. In addition, *brca2*^{lex1} mice are of normal size and fertile; unlike mice with the larger COOH-terminal truncations that retain some of the BRC motifs. Thus, the *brca2*^{lex1} mutation is very mild, and suggests only a modest impairment of Brca2 activity. However, even though *brca2*^{lex1} mice exhibit a mild phenotype, MEF bearing this mutation exhibit impaired growth, gross chromosomal instability and severe hypersensitivity to the interstrand crosslinking agent mitomycin C.

Body

1) Breeding of *brca2*^{lex1} and *brca2*^{lex2} mice. Previously we developed mouse ES cells and MEF with a compound heterozygous mutation for *Brca2* in an isogenic 129SvEv background (Morimatsu *et al.*, 1998). One mutated allele is called *brca2*^{lex1} and the other *brca2*^{lex2}. The *brca2*^{lex1} mutation is a precise deletion of exon 27, while the *brca2*^{lex2} mutation is a deletion of exon 27 and part of exon 26. The impact of these mutations was observed in mice generated from the ES cells. The mice are in a 129SvEv – C57Bl6 crossbred background. The progeny from *brca2*^{lex2} heterozygous breeding pairs were observed. Of 56 mice genotyped from *Brca2*$^{+/lex2}$ crosses, there were 32 *Brca2*$^{+/lex2}$ and 24 *Brca2*$^{+/+}$ mice. No *brca2*$^{lex2/lex2}$ mice were generated from *Brca2*$^{+/lex2}$ breeding pairs, demonstrating that the *brca2*^{lex2} mutation is an embryonic lethal. We also genotyped day 10.5 embryos from a *Brca2*$^{+/lex2}$ female crossed with a *Brca2*$^{lex1/lex2}$ male. In addition to *Brca2*$^{+/lex2}$ and *Brca2*$^{lex1/lex2}$ embryos, which were normal in appearance, one *brca2*$^{lex2/lex2}$ embryo was recovered, which was partially resorbed and appeared to have arrested at approximately embryonic day 6. This is a similarly severe phenotype as observed with truncations that delete all of the identified Rad51-interaction domains (Sharan *et al.*, 1997, Suzuki *et al.*, 1997, Ludwig *et al.*, 1997), and implies that the *brca2*^{lex2} allele is a null. It may be that the *brca2*^{lex2} mutation destabilizes the transcript; a corresponding mRNA could not be detected by RT-PCR from *brca2*$^{lex1/lex2}$ cells.

Given that the *brca2*^{lex2} mutation is an early embryonic lethal, the *brca2*^{lex1} mutation must produce a partially functional protein, since *brca2*$^{lex1/lex2}$ cells and mice are viable. The *brca2*^{lex1} mutation enables transcription because exon 27 was replaced with a splice acceptor, stop codon and polyadenylation site (part of the *Hprt* minigene positive selection cassette) (Morimatus *et al.*, 1998). The predicted fusion of *Brca2* exon 26 into the new splice acceptor was demonstrated by RT-PCR from *brca2*$^{lex1/lex2}$ cells, and yields a transcript in which the nucleotides that code for amino acids 3140-3328 are deleted (Morimatus *et al.*, 1998). Further analysis showed that a truncated protein was translated from this transcript that retains the ability to associate with Rad51 in the nucleus, presumably through the BRC motifs (Moynahan *et al.*, 2001). This interaction appears to be unperturbed in *brca2*$^{lex1/lex2}$ cells. Thus, the *brca2*^{lex1} allele produces a protein that contains more than 94% of the protein (including all the BRC motifs) and still efficiently associates with Rad51 in the nucleus. The progeny from *brca2*^{lex1} heterozygous breeding pairs were observed. There was a normal Mendelian pattern of inheritance. The *brca2*$^{lex1/lex1}$ mice did not exhibit any obvious phenotype compared to their control littermates (*Brca2*$^{+/+}$, *Brca2*$^{+/lex1}$). There was no reduction in size or fecundity. Since mice that are deleted for some, but not all, of the BRC motifs are infertile (Conner *et al.*, 1997; Friedman *et al.*, 1998), this implies that the complete complement of BRC motifs in the *brca2*^{lex1} protein is necessary and sufficient for meiotic recombination.

2) Life Span of *brca2*$^{lex1/lex1}$ and Control Mice. We compared the life span of 52 *brca2*$^{lex1/lex1}$ mice to 35 control mice (Figure 1b). Included in the control cohort are *Brca2*$^{+/+}$, *Brca2*$^{+/lex1}$, and *Brca2*$^{+/lex2}$ mice. Heterozygote mice were included because there is little phenotypic abnormality in these mice.

Mortality came sooner for the *brca2*$^{lex1/lex1}$ mice compared to control mice. The onset of mortality is about the same for *brca2*$^{lex1/lex1}$ and control mice; 50 - 60 weeks. However, the life span curves began to diverge at about 70 weeks and were significantly different by 93 weeks ($p < 0.1$). This divergence progressed until the study was terminated, when the youngest mice were 108 weeks, ($p < 0.01$). About 50% of the *brca2*$^{lex1/lex1}$ mice die by 89 - 90 weeks while 50% of the control mice die by 104 - 108 weeks.

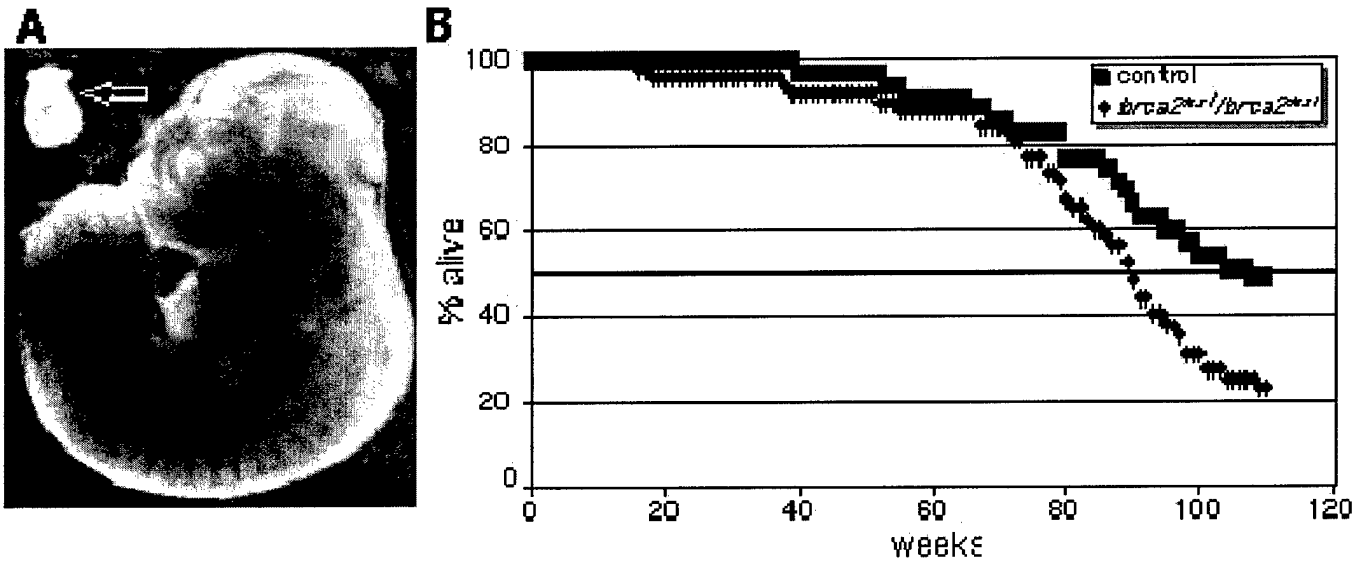

Figure 1. The *brca2*^{lex1} and the *brca2*^{lex2} mutations in mice. **A.** Day E10.5 embryos harvested from a *brca2*$^{+/lex2}$ female mated with a *brca2*$^{lex1/lex2}$ male. The larger embryo is *brca2*$^{lex1/lex2}$ compound heterozygote and appears morphologically normal. The much smaller (partially resorbed) embryo indicated by the arrow at left is a *brca2*$^{lex2/lex2}$ homozygote. **B.** Survival curve of *brca2*$^{lex1/lex1}$ (blue diamonds) and control (red squares) mice. The percent alive are shown.

3) Pathology of *brca2*$^{lex1/lex1}$ and Control Mice. Moribund and recently dead mice were observed by necropsy. Additionally, all remaining mice were sacrificed at the point when the survival curve reached 49% for the control population. Abnormal tissues and potential tumors were examined by histopathology, as summarized in Table 1. Both *brca2*$^{lex1/lex1}$ and control mice exhibit a wide range of cancers. However, onsets of cancer and sepsis (the latter indicated by reactive immune responses) were somewhat earlier in the *brca2*$^{lex1/lex1}$ mice. From weeks 67 to 104, 11 *brca2*$^{lex1/lex1}$ mice (~20% of the population) developed life-threatening pathology (either cancer or sepsis) while at week 104 the first control mouse exhibited life-threatening disease. The average age of onset for frank neoplasias, considering only tumors that arose through 108 weeks, was 81 weeks for the *brca2*$^{lex1/lex1}$ mice as compared to 106 weeks for controls ($p < .005$). The cumulative incidence of cancers and reactive immune responses was significantly higher in the *brca2*$^{lex1/lex1}$ cohort compared to the control cohort by week 87 and continued to diverge through 108 weeks (p values from <.05 to .01). Since many of the *brca2*$^{lex1/lex1}$ mice were generated after the control mice, not as many *brca2*$^{lex1/lex1}$ mice as controls were observed at ages older than 110 weeks (9 compared to 16) or older than 120 weeks (1 compared to 7). For this reason, the frequency of late onset disease is not known for the *brca2*$^{lex1/lex1}$ mice and cannot be compared to control mice. Moreover, the differences seen through 108 weeks, while statistically significant, should be interpreted with caution given the small numbers of animals involved.

Nonetheless, the shortened life span observed in the *brca2*$^{lex1/lex1}$ cohort appears to be due, at least in part, to early onsets of cancer and sepsis.

mouse	genotype	week	histopathology
284H	lex1/lex1	67	messenteric abscess
246H	lex1/lex1	77	colonic adenocarcinoma
107H	lex1/lex1	79	hepatocellular carcinoma
109H	lex1/lex1	82	fatty liver with focal inflamation
103H	lex1/lex1	86	granulamatous inflamation in spleen
264H	lex1/lex1	87	malignant lymphoma
164H	lex1/lex1	90	reactive lymphoid hyperplasia in spleen
094H	lex1/lex1	96	granulamatous inflamation in lymph nodes and skin
281H	lex1/lex1	98	massive lymphoid hyperplasia in lymph nodes, possibly lymphoma
267H	lex1/lex1	101	reactive lymphoid hyperplasia in lymph nodes, spleen, liver, kidney
050H	lex1/lex1	104	hepatocellular carcinoma
256H	lex1/lex1	114	osteosarcoma
022N	+/lex2	104	hepatocellular carcinoma
028H	+/+	108	leimyosarcoma
051H	+/lex1	116	hemangiosarcoma
053H	+/lex1	116	hepatocellular carcinoma
058H	+/lex1	119	malignant lymphoma
049H	+/lex1	129	hepatocellular carcinoma

Table 1. Pathology of *brca2*$^{lex1/lex1}$ and Control Mice.

4) Immortalized *brca2*$^{lex1/lex2}$ MEF show reduced cellular viability. Previous observations of *brca2*$^{lex1/lex2}$ ES cells and MEF suggest that the shortened life span of *brca2*$^{lex1/lex1}$ mice and early onset of cancer/sepsis might be related to inefficient recombinational repair and resulting genomic instability (Morimatus *et al.*, 1998; Moynahan *et al.*, 2001). In order to assess the cellular phenotype conferred by the *brca2*^{lex1} allele in greater depth, further studies were performed on immortalized *brca2*$^{lex1/lex2}$ and control MEF. Wild type and *brca2*$^{lex1/lex2}$ ES cells (AB2.2 cells from 129SvEv mice) were used to generate populations of primary MEF cells, and hence immortalized MEF, as previously described (Marimatus *et al.*, 1998). The primary *brca2*$^{lex1/lex2}$ MEF underwent premature replicative senescence in culture and, perhaps because of this growth defect, it was more difficult to derive spontaneously immortalized *brca2*$^{lex1/lex2}$ MEF than wild type MEF; only a single clone was obtained. The immortalized *brca2*$^{lex1/lex2}$ MEF could be propagated readily, but their growth was slower than that of isogenic wild type immortalized cells. To determine whether cellular viability is impaired in the immortalized *brca2*$^{lex1/lex2}$ MEF cells, their cloning efficiency was measured by colony formation assays. In eleven paired determinations, mean cloning efficiency for wild type MEF was 24.9% (S.D. = 10.9%), but for the immortalized *brca2*$^{lex1/lex2}$ MEF, only 16.0% (S.D. = 4.8%), i.e. reduced by about one third. Thus, although replicative senescence has been overcome in the immortalized *brca2*$^{lex1/lex2}$ MEF, it appears that the original growth defect has not been entirely suppressed, as cloning efficiency is still significantly reduced relative to wild type (t-test; $p < 0.002$).

5) Immortalized *brca2*$^{lex1/lex2}$ MEF are moderately hypersensitive to ionizing radiation. We have shown previously that *brca2*$^{lex1/lex2}$ ES cells are more sensitive than wild-type ES cells to □-radiation,

suggesting a defect in repair of DNA double-strand breaks (Morimatsu *et al.*, 1998). To determine whether immortalized *brca2*$^{lex1/lex2}$ MEF retain this phenotype, their sensitivity was assessed in comparison to immortalized isogenic wild type MEF. Sensitivity was measured by survival (colony formation) after acute exposure to □-radiation at doses ranging from 2 to 10 Gy. At each dose tested, *brca2*$^{lex1/lex2}$ MEF were significantly more sensitive than wild type MEF (p values ranging from 0.01 to 0.05). At 2 Gy, survival of *brca2*$^{lex1/lex2}$ MEF was reduced by only 1.5 - fold relative to wild type. However, the difference became greater with increasing doses, so that at 10 Gy, survival of *brca2*$^{lex1/lex2}$ MEF was reduced by 10-fold relative to wild type. Compared in terms of the dose required to reduce viability to 10% of non-irradiated controls, *brca2*$^{lex1/lex2}$ MEF were about 1.4-fold more sensitive to □-radiation than wild-type MEF. Both *brca2*$^{lex1/lex2}$ and wild type MEF appear to be more resistant to □-radiation than the corresponding ES cell lines. Nonetheless, the relative difference in sensitivity between *brca2*$^{lex1/lex2}$ cells and wild type cells, in terms of dose required to reduce survival by 90%, is comparable: about 1.4-fold for the immortalized MEF cells, and about 1.7-fold for the ES cells.

6) **Immortalized *brca2*$^{lex1/lex2}$ MEF are severely hypersensitive to mitomycin C.** Extreme hypersensitivity to drugs that cause DNA interstrand crosslinks has been observed in mammalian cell lines defective in recombinational repair: irs1 cells mutant for *XRCC2*; irsSF cells mutant for *XRCC3* (Caldecott *et al.*, 1991; Tebbs *et al.*, 1995; Cui *et al.*, 1999), and also in cells with a long COOH-terminal truncation in *Brca2* that removed some of the BRC motifs (Yu *et al.*, 2000). We compared immortalized *brca2*$^{lex1/lex2}$ MEF to immortalized wild type MEF for sensitivity to the DNA crosslinking drug mitomycin C. Sensitivity was measured by survival and proliferation (colony formation) after plating in the presence of mitomycin C at initial concentrations ranging from 3×10^{-9} M to 3×10^{-7} M. Mean survival after plating in mitomycin C at 3×10^{-9} M (or less) was not significantly lower for *brca2*$^{lex1/lex2}$ MEF than for wild-type MEF. But at progressively higher concentrations of mitomycin C (1×10^{-8} M, 3×10^{-8} M and 1×10^{-7} M), survival of *brca2*$^{lex1/lex2}$ MEF fell sharply and was significantly lower than for wild-type MEF ($P < 0.02$, < 0.005 and < 0.01, respectively). At 3×10^{-8} M mitomycin C, survival of *brca2*$^{lex1/lex2}$ MEF was 5.6-fold lower, and at 1×10^{-7} M, 48-fold lower than for wild type MEF. Compared in terms of the concentration of mitomycin C required to reduce survival to 50% of nontreated controls, *brca2*$^{lex1/lex2}$ MEF were approximately eight-fold more sensitive than wild type MEF.

7) To assess the relative effects of DNA damage on chromosomal integrity in wild type and *brca2*$^{lex1/lex2}$ immortalized MEF. Metaphase spreads were prepared 24 hours after exposure to 6 Gy of □-radiation or to mitomycin C at 5×10^{-8} M. After □-irradiation, there were significantly increased numbers of chromatid- and chromosome-type abnormalities in *brca2*$^{lex1/lex2}$ MEF relative to non-irradiated controls, but this was also true for wild type MEF (See Appendix – Table2). The numbers of chromatid and chromosome aberrations present in *brca2*$^{lex1/lex2}$ MEF after irradiation were only slightly higher than in wild type MEF. This is consistent with the modest difference between *brca2*$^{lex1/lex2}$ and wild type cells in viability after □-irradiation, and indicates that *brca2*$^{lex1/lex2}$ cells have nearly the proficiency of wild-type cells in repairing the DNA damage induced by ionizing radiation. Mitomycin C at 5×10^{-8} M produced no significant increase in chromatid- or chromosome-type abnormalities in wild type MEF (Table 2), suggesting that cells expressing normal Brca2 were able to repair most of the interstrand crosslinks created. In *brca2*$^{lex1/lex2}$ MEF, however, the frequency of chromatid-type aberrations increased more than two-fold after mitomycin C exposure (Table 2. $p < 0.001$).

We wished to determine whether the chromosomal instability seen in immortalized *brca2*$^{lex1/lex2}$ MEF arose during the process of immortalization, or already existed in primary cells prior to senescent crisis. We therefore examined early-passage primary MEF for chromosomal abnormality. Two populations of *brca2*$^{lex1/lex2}$ MEF and three populations of wild type MEF (Morimatsu *et al.*, 1998) that had been frozen at the first passage after dissociation from embryos (passage 1) were thawed and passaged, with metaphase spreads prepared at each passage. Passage 1 wild-type MEF grew vigorously after thaw and showed no appreciable slowing of growth over at least ten passages afterward (data not shown). However, the *brca2*$^{lex1/lex2}$ MEF senesced prematurely in culture, as reported previously (Morimatsu *et al.*, 1998). and could not be expanded beyond the fourth passage. For one of the two primary *brca2*$^{lex1/lex2}$ MEF populations examined (281.1), sufficient numbers of metaphase cells for scoring were recovered only at the second and third passages. Scoring of early passage primary MEF for chromosomal aberrations is summarized in Appendix – Table3. Compared to wild type primary MEF, the *brca2*$^{lex1/lex2}$ primary MEF had sharply elevated numbers of chromatid and chromosome aberrations, including gaps, breaks, deletions and exchanges. The *brca2*$^{lex1/lex2}$ MEF had higher numbers of chromosomal aberrations even at passage 2, and accumulated further aberrations more rapidly than wild type MEF. By the third passage, approximately half the metaphase cells in *brca2*$^{lex1/lex2}$ populations had one or more visible chromosome abnormalities. These results confirm that the chromosomal instability seen in immortalized *brca2*$^{lex1/lex2}$ MEF results directly from the *brca2*$^{lex1/lex2}$ mutation, and not from additional mutations acquired during or after immortalization.

Conclusion and Summary

The primary BRCA2 $^{lex1/lex2}$ MEF cells previously reported have been used to derive spontaneously immortalized MEF. Here we report characterization of these cells for sensitivity to mitomycin C, which produces DNA interstrand crosslinks; a type of damage thought to require repair through homologous recombination. We find that deletion of the BRCA2 exon 27 Rad51-binding domain results in a marked hypersensitivity to mitomycin C in immortalized MEF cells, indicating a defect in ability to repair DNA interstrand crosslinks. Because mutations that impair homologous recombination have been associated with chromosomal instability, in yeast and in mammalian cells, we examined primary and immortalized BRCA2 $^{lex1/lex2}$ MEF for effects of the mutation on chromosome stability. Both immortalized and early-passage primary MEF cells deleted for the exon 27 RAD51-binding domain also exhibit severe chromosomal instability.

The results obtained here confirm that the extreme COOH-terminal region encoded by exon 27, though not essential to viability, is important for the function of Brca2 at the cellular level. It remains a possibility that the phenotypic effects associated with deletion of exon 27 result from something other than altered interactions with Rad51. In humans, the Brca2 protein is dependent upon nuclear localization signals encoded within exon 27 for transport into the nucleus (Spain *et al.*, 1999). However, this is evidently not the case for mouse Brca2, since the protein encoded by the *brca2*^{lex1} allele reaches the nucleus (Moynahan *et al.*, 2001). Thus, in mouse, interaction with Rad51 is the only function so far demonstrated for the region encoded by exon 27. This raises the interesting question of why the Brca2 protein should require multiple Rad51 interaction domains (the BRC repeats as well as the region encoded by exon 27) to be fully functional.

Although the cellular phenotypes of the brca2^{lex1} truncation and the longer COOH-terminal Brca2 deletions are similar, the mouse phenotypes are very different. Mice homozygous for either of the long COOH-terminal deletions exhibit partial pre-adulthood lethality, infertility, runted growth and high incidence of thymic lymphoma before six months of age (Connor *et al.*, 1997; Patel *et al.*, 1998; Friedman *et al.*, 1998). However, mice that are homozygous for the *brca2*^{lex1} truncation appear relatively normal. They grow, reach adulthood, and are fertile much like their control littermates. Nearly all the mice survive beyond one year of age, yet their life span is significantly shorter than control mice, possibly due to early onsets of cancer and sepsis. That the larger COOH-terminal deletions have severely deleterious phenotypes is perhaps unsurprising, given that they eliminate more than a third (Connor *et al.*, 1997), or more than half (Patel *et al.*, 1998) of the Brca2 protein. The large COOH-terminal regions lost in these mutations are likely to contain additional domains with important functions, and so the resulting phenotypes cannot be ascribed with certainty to the loss of Rad51-interacting regions alone. The *brca2*^{lex1} allele deletes less than 6% of the protein and leaves all eight of the BRC repeats, as well as the intervening region coded by exons 12 – 26 intact. Moreover, the truncated Brca2 protein encoded by the *brca2*^{lex1} allele retains the capacity to bind Rad51, presumably via the BRC repeats (Moynahan *et al.*, 2001). Nonetheless, the *brca2*^{lex1} allele produces a qualitatively similar cellular phenotype as the larger deletions. Thus, while the more severe phenotypes seen in mice with longer COOH-terminal deletions may be the result of more severely impaired HRR, it is also possible that they reflect loss of functions unrelated to Rad51. Due to this possibility, the *brca2*$^{lex1/lex1}$ mice are an ideal genetic background to evaluate mutations in other genes suspected to functionally interact with Brca2. They may also have utility in testing putative clastogens or carcinogens, since they are predisposed to genetic instability, but exhibit such a mild phenotype.

Key Researech Accomplishments

1. The BRCA2^{lex2} allele is embryonic lethal when homozygous.
2. Immortalized BRCA2 $^{lex1/lex2}$ MEF are moderately hypersensitive to ionizing radiation.
3. Immortalized BRCA2 $^{lex1/lex2}$ MEF are severely hypersensitive to mitomycin C.
4. Mice homozygous for either of the long COOH-terminal deletions exhibit partial pre-adulthood lethality, infertility, runted growth and high incidence of thymic lymphoma before six months of age.
5. BRCA2$^{lex1/lex2}$ MEF exhibit chromosomal instability

Reportable Outcomes

1. A manuscript entitled "A short COOH-terminal deletion of BRCA2 in mice causes chromosomal instability, increases sensitivity to DNA interstrand crosslinks and shortens life span" was in preparation and is close to submit for publication.
2. We have generated mouse immortalized BRCA2$^{lex1/lex2}$ MEF.
3. A postdoctoral fellow, Dr. Mark Breenman was hired for this study.

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Appendix:

Table 2. Spontaneous and damage-induced chromosomal aberrations in immortalized wild type and brca2$^{lex1/lex2}$ MEF cells.

Table 3. Spontaneous chromosomal aberrations in early-passage wild type and Brca2$^{lex1/lex2}$ primary MEF cells.

Table 2. Spontaneous and damage-induced chromosomal aberrations in immortalized wild type and brca2$^{lex1/lex2}$ MEF cells.

MEF cells	Metaphases analyzed	Percent aberrant metaphases[3]	Mean aberrations per metaphase[3]	Chromatid aberrations[1]				Chromosome aberrations[2]			
				gaps	breaks	other	total[3]	int.del.	ter.del.	other	total
Wild type											
No treatment	30	50	0.800	5	9	2	11	9	3	1	13
γ-irradiation	30	100	4.33	10	25	29	54	65	5	5	77
Mitomycin C	30	60	0.844	0	8	6	14	8	5	0	13
brca2$^{lex1/lex2}$											
No treatment	30	97	3.27	7	24	17	41	47	10	0	57
γ-irradiation	30	100	5.07	17	35	24	59	75	0	18	93
Mitomycin C	30	93	4.60	13	66	30	96	27	14	1	42

1. "gaps" defined as discontinuities smaller than the width of the chromatid; "other" comprising isochromatid deletions and exchanges.
2. interstitial deletions; terminal deletions (including breaks); and other (including dicentric and ring chromosomes).
3. calculated without chromatid gaps.

Table 3. Spontaneous chromosomal aberrations in early-passage wild type and Brca2$^{lex1/lex2}$ primary MEF cells.

MEF cells	Passage	Metaphases analyzed	Percent aberrant metaphases[3]	Mean aberrations per Metaphase[3]	Chromatid aberrations[1]				Chromosome aberrations[2]			
					gaps	breaks	other	total[3]	int.del.	ter.del.	other	total
Wild type												
129.5	P2	50	6	0.06	7	1	0	1	0	2	0	2
	P3	50	6	0.06	13	1	0	1	0	1	1	2
	P4	50	10	0.10	10	1	0	1	0	2	2	4
129.7	P2	50	4	0.06	4	3	0	3	0	0	0	0
	P3	50	8	0.12	1	1	1	2	2	2	0	4
	P4	50	14	0.24	3	5	0	5	2	5	0	7
129.9	P2	50	4	0.04	0	0	0	0	0	2	0	2
	P3	50	8	0.08	1	2	0	2	0	2	0	2
	P4	50	26	0.28	7	3	2	5	3	5	1	9
Brca2$^{lex1/lex2}$												
283.2	P2	50	24	0.40	22	11	2	13	0	7	0	7
	P3	50	58	2.22	22	50	6	56	23	32	0	55
	P4	42	48	1.02	14	18	4	22	2	27	0	29
281.1	P2	50	52	0.76	5	23	0	23	5	10	0	15
	P3	40	62	1.60	23	35	5	40	5	19	0	24

1. "gaps" defined as discontinuities smaller than the width of the chromatid; "other" comprising isochromatid deletions and exchanges.
2. interstitial deletions; terminal deletions (including breaks); and other (including dicentric and ring chromosomes).
3. calculated without chromatid gaps.