Role for the BRCA1 C-terminal Repeats (BRCT) Protein 53BP1 in Maintaining Genomic Stability*

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53BP1 that includes a recently described kinetochore-binding domain (28) is necessary for the formation of irradiation-induced p53/MDM2 nuclear foci and physically interacts with 53BP1 (20, 24). Recent studies have revealed a role for 53BP1 in cell cycle checkpoints (25–27) as well as in maintaining p53 levels in response to γ-IR (27). Here we show that a 380-amino-acid region of 53BP1 that includes a recently described kinetochore-binding domain (28) is necessary for the formation of irradiation-in-

53BP1 is phosphorylated in response to DNA damage and rapidly relocates to presumptive sites of DNA damage along with Mre11 and the phosphorylated histone 2A variant, γ-H2AX. 53BP1 associates with the BRCA1 tumor suppressor, and knockdown experiments with small interfering RNA have revealed a role for the protein in the checkpoint response to DNA damage. By generating mice defective in m53BP1 (m53BP1tettra), we have created an animal model to further explore its biochemical and genetic roles in vivo. We find that m53BP1tetra animals are growth-retarded and show various immune deficiencies including a specific reduction in thymus size and T cell count. Consistent with a role in responding to DNA damage, we find that m53BP1tetra mice are sensitive to ionizing radiation (γ-IR), and cells from these animals exhibit chromosomal abnormalities consistent with defects in DNA repair. Thus, 53BP1 is a critical element in the DNA damage response and plays an integral role in maintaining genomic stability.

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DNA damage-response mechanisms ensure the fidelity of chromosomal transmission, and their failure may lead to the development of diseases such as cancer (1). In response to γ-IR, phosphoinositide-like kinases (PIKs) such as ATM (mutated in ataxia-telangiectasia) transduce damage signals to kinases, transcription factors, and DNA repair proteins by targeting (S/T)Q motifs (2). A second PIK, ATR (ATM- and Rad3-related), also responds to γ-IR, but it appears to respond primarily to agents that create replicational stress (i.e. hydroxyurea and aphidicolin) (2). ATM and ATR have distinct but overlapping substrate specificities including the ability of both enzymes to target p53 serine residue 15 (Ser-15) as well as the product of breast cancer susceptibility gene 1, BRCA1, at Ser-1423 (3, 4). BRCA1 is a major target of the DNA damage response, and mutations in BRCA1 contribute to nearly 50% of familial forms of breast and ovarian cancer (5). BRCA1 had been found associated with RNA polymerase II (6), chromatin-remodeling factors (7), and a variety of DNA repair and replication factors (8–10). Indeed, BRCA1 has been shown to function in genomic stability by controlling homologous recombination, transcription-coupled repair of oxidative DNA damage, and cell cycle checkpoints (11–14).

One protein that contains numerous (S/T)Q motifs and two C-terminal BRCT repeats is p53-binding protein 1 (53BP1). 53BP1 was discovered as a p53-interacting factor in a two-hybrid screen (15) and was subsequently proposed to function as a transcriptional co-activator of p53 (16). Although the relationship between 53BP1 and p53 has not been fully established, 53BP1 and p53 from both Xenopus and humans have been shown to interact either directly or indirectly in experimental settings that express high levels of 53BP1 protein from plasmids or that naturally occur in eggs (15, 17). We, as well as others, have demonstrated previously that 53BP1 is involved in the DNA damage-response network (17–20). 53BP1 proteins are phosphorylated in response to γ-IR, and this is likely governed by the action of PIKs like ATM (17, 19, 20). γ-IR also induces 53BP1 to rapidly relocalize to DNA repair foci, and this response is delayed or inhibited by treatment with the PIK inhibitors caffeine and wortmannin. 53BP1 foci also overlap with those formed by the Mre11 complex, BRCA1, and the phosphorylated form of the histone variant H2AX (γ-H2AX; see Refs. 18–20). As both the Mre11 complex and γ-H2AX are believed to localize to physical sites of DNA damage (21–23) and to recruit various DNA repair factors to these sites, 53BP1 has been inferred to localize to these sites as well. This notion is further supported by the fact that γ-H2AX recruits 53BP1 to nuclear foci and physically interacts with 53BP1 (20, 24). Recent studies have revealed a role for 53BP1 in cell cycle checkpoints (25–27) as well as in maintaining p53 levels in response to γ-IR (27). Here we show that a 380-amino-acid region of 53BP1 that includes a recently described kinetochore-binding domain (28) is necessary for the formation of irradiation-in-
denced foci. We further deciphered the role of 53BP1 in the DNA damage response by generating mice defective in m53BP1. We report that murine animals expressing a truncated form of m53BP1 (m53BP1<sup>1/2tr</sup>) exhibit a pleiotropic phenotype that includes growth retardation, immune deficiencies including defects in T cell maturation, sensitivity to γ-IR, as well as increased chromosomal aberrations. Taken together, these results reveal that 53BP1 is an integral component of the DNA damage-response network and indicate that the protein plays an important role in maintaining genomic stability.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Indirect Immunofluorescence**—Three antibodies that recognize both the human and murine 53BP1 proteins were generated for this study. We found that our 53BP1 antibodies recognize both the murine and human proteins. Polyclonal antibodies raised against glutathione S-transferase fusion proteins encoding the first 524 amino acids of human 53BP1 (α53BP1) or the last 200 residues of the protein (α53BP1-C) were affinity-purified by established procedures and used as described in the text. α3BP1-N is a polyclonal, anti-peptide antibody that was raised against an N-terminal sequence GVELEQSQQD-VPE that is conserved between human and murine 53BP1 proteins. Polyclonal antibodies were affinity-purified by standard methods. Anti-HA antibodies were purchased from Covance, and anti-ATR antibodies were obtained from Oncogene Research Products.

**Mouse Genetics and Genotyping of m53BP1<sup>1/2tr</sup> Animals**—Murine animals defective in m53BP1 (m53BP1<sup>1/2tr</sup>) were generated with a random XbaI site but upstream of the 3′-proximal, 1.5-kb fragment. To detect this fragment, we PCR-amplified and labeled with <sup>32</sup>P a probe downstream of the 5′-terminally occurring XbaI site but upstream of the one introduced by VICTR54, as shown in Fig. 2A. The primers used to amplify the 700-base pair probe for Southern analysis were 5′-CTCGACATCCATGCTGGGC-3′ and 5′-TACTTAAAGGACTCTGACACGC-3′. The sequences of the primers used for RT-PCR analysis were as follows: A, 5′-CTCTTGCTGCAGACACAG-3′; B, 5′-CTGCGGTGTCGCTGTCCGGCGAGACTC-3′; C, 5′-GTCGCGGTGTCGCTGTCCGGCGAGACTC-3′; D, 5′-GTCGCGGTGTCGCTGTCCGGCGAGACTC-3′. Either poly(A)<sup>+</sup> or total RNA was isolated by standard methods and used to prepare cDNA with the Superscript one-step PCR system (Invitrogen).

**Immune System Analysis**—Bone marrow, thymus, and spleen tissue were prepared as described previously (29). Genomic DNA was isolated from mouse tail snips by standard methods. Insertion of VICTR54, as described previously (29). Genomic DNA was isolated 14972

**RESULTS AND DISCUSSION**

**Dynamic Nuclear Localization of 53BP1 in the Absence and Presence of DNA Damage**—It has been recently shown that 53BP1 localizes to the kinetochore during mitosis (28). However, the behavior of 53BP1 during interphase in the absence of extrinsic DNA damage has not been fully investigated. To examine the interphase behavior of 53BP1 during the course of normal, unperturbed MCF-7 cell cycles, we used a laser scanning confing cytometer to determine the nuclear localization of 53BP1 and the cellular DNA content for any given cell. In G<sub>1</sub>, 53BP1 exists in a diffuse nuclear pattern as well as in large nuclear “dots” (Fig. 1A) that are absent in S-phase, 53BP1 can be found in a discrete, punctate pattern (Fig. 1A). The nuclear distribution pattern of 53BP1 in G<sub>1</sub> cells appeared in two types, one similar to S-phase but with fewer foci (Fig. 1A) and one that exhibited few, if any, large dots (not shown). It is well established that 53BP1 relocates to nuclear foci in response to DNA damage (17–20). We found that 53BP1 and ATR co-localized to nuclear foci in response to hydroxyurea (Fig. 1B). We also found that 53BP1 physically associates with ATR in nuclear extracts derived from K562 cells (Fig. 1C). 53BP1 can be detected in ATR immunoprecipitates and ATR is present in 53BP1 immunoprecipitates, and the association occurs independently of DNA damage (Fig. 1C). Moreover, ATR phosphorylates 53BP1 in vitro (not shown). Thus, 53BP1 interacts with various factors implicated in genomic stability including ATR, p53, H2AX, BRCA1, and Chk2. To address which structural elements of 53BP1 are required for the formation of irradiation-induced foci, we created a series of mutant constructs in the 53BP1-expression vector pCMH6K53BP1 (16). We generated mutant forms of 53BP1 that deleted the C-terminal BRCT motifs (ABRCT), the kinetochore-binding region (∆KINET), the N-terminal, 1,234 residues except for the initiation codon (∆N3H), and a protein mutated at 15 potential phosphorylation sites (15AQ), some of which are known to be targeted during the DNA damage response. All constructs maintained the nuclear localization signal. Transfected with these various types, we observed that MCF-7 cells revealed that the mutant proteins were being expressed (not shown). We confirmed that the wild-type, HA-tagged version of 53BP1 encoded by pCMH6K53BP1 generated nuclear foci in response to DNA damage when immunostained with an antibody specific for the HA tag (Fig. 1D). Untransfected cells were found to stain negative with anti-HA antibodies (not shown). Our results indicate that the majority of 53BP1 appears dispensable for DNA damage-induced nuclear foci formation, including the N-terminal 1,234 residues (which includes numerous S/T/Q motifs) as well as the C-terminal BRCT motifs (Fig. 1D). Surprisingly, ∆KINET, a 380-amino-acid deletion (residues 1,236–1,615) that removes the kinetochore binding region (28) of 53BP1, failed to form irradiation-induced foci as the protein persisted in a diffuse nuclear pattern after irradiation (Fig. 1D).

**Generation of Mice Defective in m53BP1 (m53BP1<sup>1/2tr</sup>)**—To begin to decipher the functional role for 53BP1 in the DNA damage response, we identified embryonic stem cells (OST94324) from Omnnbank (29) containing a single, −5.0-kb retroviral insertion (VICTR54; Fig. 2A) in murine 53BP1 (m53BP1; 1,957 amino acids; 80% identity to human 53BP1; see Ref. 28). VICTR54 was found inserted within a 4.9-kb intron located between exons 13 and 14 (Fig. 2A). VICTR54, and its related vectors, are usually found within introns and contain splice acceptor (SA) and donor (SD) sequences such that a neomycin (NEO) resistance gene and flanking sequences are spliced into the mature transcript as an exon (Fig. 2A) (29). These transcriptional fusions disrupt the coding sequence through the introduction of premature stop codons. Such gene trapping methodologies have been applied previously to understanding gene function (29). OST94324 cells were used to gen-

<sup>2</sup>Z. Xia, J. C. Morales, and P. B. Carpenter, unpublished data.
erate transgenic animals heterozygous in m53BP1 (m53BP1+/tr) as described previously (29). Southern blotting with DNA isolated from tail biopsies confirmed the disruption in m53BP1 and was used to genotype the animals (Fig. 2B; see “Experimental Procedures”). Crosses between heterozygous animals produced m53BP1+/tr progeny born at the expected frequencies. The m53BP1+/tr animals were found to be fertile, but we did observe that crosses between mutant animals produced smaller litters as some embryos spontaneously aborted and were reabsorbed by the mother (data not shown). RT-PCR analysis with various primers 5′ and 3′ to the insertion demonstrated that exon 13 failed to properly splice next to exon 14 in the m53BP1+/tr mice (Fig. 2C). Rather, the “artificial” exon containing neomycin from VICTR54 was spliced adjacent to exon 13 as verified with primers specific for exon 13 and the neomycin gene (primer set D/A; Fig. 2C). Sequencing of a cloned RT-PCR product spanning the insertion event revealed that the natural coding sequence of m53BP1 had stopped after residue 1,205, where it then fused to 21 residues derived from NH3 (deletes the first 1,234 amino acid residues except for the initiation codon), and 2BRCT (deletes amino acid residues 1,236–1,615), ΔNLS (deletes the first 1,234 amino acids residues except for the initiation codon), and 15AQ, a construct with mutations in 15 (S/T)Q sites. The following serine or threonine residues were mutated to alanines specific for the HA tag (Covance). The following constructs expressing in-frame 53BP1 deletions or mutations were made: ΔNLS (deletes amino acid residues 1,786–1,964), ΔKINET (deletes amino acid residues 1,236–1,615), ΔBRCT (deletes the first 1,234 amino acids residues except for the initiation codon), and 15AQ, a construct with mutations in 15 (S/T)Q sites. The following serine or threonine residues were mutated to alanines specific for the HA tag (Covance). The following constructs expressing in-frame 53BP1 deletions or mutations were made: ΔNLS (deletes amino acid residues 1,786–1,964), ΔKINET (deletes amino acid residues 1,236–1,615), ΔBRCT (deletes the first 1,234 amino acids residues except for the initiation codon), and 15AQ, a construct with mutations in 15 (S/T)Q sites. The following serine or threonine residues were mutated to alanines specific for the HA tag (Covance). The following constructs expressing in-frame 53BP1 deletions or mutations were made: ΔNLS (deletes amino acid residues 1,786–1,964), ΔKINET (deletes amino acid residues 1,236–1,615), ΔBRCT (deletes the first 1,234 amino acids residues except for the initiation codon), and 15AQ, a construct with mutations in 15 (S/T)Q sites. The following serine or threonine residues were mutated to alanines specific for the HA tag (Covance). The following constructs expressing in-frame 53BP1 deletions or mutations were made: ΔNLS (deletes amino acid residues 1,786–1,964), ΔKINET (deletes amino acid residues 1,236–1,615), ΔBRCT (deletes the first 1,234 amino acids residues except for the initiation codon), and 15AQ, a construct with mutations in 15 (S/T)Q sites. The following serine or threonine residues were mutated to alanines specific for the HA tag (Covance). The following constructs expressing in-frame 53BP1 deletions or mutations were made: ΔNLS (deletes amino acid residues 1,786–1,964), ΔKINET (deletes amino acid residues 1,236–1,615), ΔBRCT (deletes the first 1,234 amino acids residues except for the initiation codon), and 15AQ, a construct with mutations in 15 (S/T)Q sites.
FIG. 2. Generation and characterization of mice defective in m53BP1 (m53BP1tr/tr). A, schematic diagram of insertion event in m53BP1 (not drawn to scale). The thick horizontal lines represent positions of probes for Southern blotting as described in B. Arrows represent the position and orientation of PCR primers used in C. The insertion of VICTR54 was determined by DNA sequencing to reside within the intron preceding exon 14 at nucleotide position 1,730 (marked by *). Splicing of the neomycin gene and flanking DNA produces a transcript that potentially disrupts the proper splicing of exons 13 and 14. LTR, long-terminal repeat; NEO, neomycin resistance gene; PGK, phosphoglycerate kinase-1; BTK, Bruton's tyrosine kinase; SA and SD, splice acceptor and donor, respectively. 

B, top, Southern blotting to determine the genotype of m53BP1-defective animals. 10 μg of genomic DNA was digested with XbaI and was probed with a radiolabeled fragment (see "Experimental Procedures") capable of discerning wild-type (WT) and mutant alleles as discussed under "Experimental Procedures." Bottom, a 700-bp probe derived from the neomycin gene was used to help genotype the animals. /H11001, wild type; /H11001/tr, heterozygous; tr/tr, homozygous. As shown in C, RT-PCR analysis indicates that improper splicing occurs between exons 13 and 14 in m53BP1tr/tr mice. Positions and orientation of primers for PCR are indicated in A. Control reactions without reverse transcriptase showed essentially no amplified products (not shown). As shown in D, m53BP1tr encodes a truncated protein of 1,226 amino acids. RT-PCR products derived from primer set A/D (as shown in C) using RNA isolated from m53BP1tr/tr animals as template were cloned into the TA vector (Invitrogen). DNA sequencing and conceptual translation indicated that m53BP1tr/tr animals potentially encode a truncated m53BP1 protein (m53BP1tr) of 1,205 natural residues along with an additional 21 residues derived from the VICTR54 vector. m53BP1 and Genomic Stability
with a variety of markers (e.g., B220, Gr-1, CD11a, and Ter119) revealed that bone marrow pro-B, pre-B, myeloid, and erythroid progenitor populations were normal in m53BP1tr/tr mice (not shown). Although CD4 and CD8 T cell populations were proportionately similar in m53BP1tr/tr and m53BP1+/+ thymuses, we observed that progression out of the DNIII stage of early thymocyte development was impaired in m53BP1tr/tr animals (Fig. 3C), the stage at which β-gene rearrangement occurs. This indicates that m53BP1 participates in proper T cell development, a process known to require various DNA repair factors (30). We also found that spleens derived from m53BP1tr/tr animals were similar in size and organ architecture to those from m53BP1+/+ animals and that the lack of functional m53BP1 did not affect the proportions of B and T lymphocytes (data not shown). We did observe, however, that m53BP1tr/tr spleens were deficient in mature B cells (IgM+IgD−; Fig. 4D), suggesting that deficiencies in m53BP1 may also result in defective B lymphocyte development.

**Genomic Instability in m53BP1tr/tr Mice**—Mice with defects in double-stranded break repair are highly sensitive to γ-IR. To evaluate whether m53BP1 contributes to increased sensitivity to DNA damage, we treated m53BP1tr/tr or wild-type animals with 7 Gy of γ-IR. After this whole body irradiation treatment, we found that 100% of the mutant animals died between 9 and 15 days post-irradiation in contrast to only 16% of the control littermates (Fig. 4A). This shows that animals defective in m53BP1 are highly sensitive to γ-IR, a result that parallels previous observations with H2AX-deficient mice (24). Despite this, we found that m53BP1tr/tr animals treated with lower doses of γ-IR (1.5 Gy) remained viable (Fig. 4B). To further explore m53BP1 function, we generated embryonic fibroblasts (MEFs) from wild-type and m53BP1tr/tr animals. m53BP1tr/tr MEFs proliferated more slowly than their wild-type counterparts (Fig. 5A). Immunofluorescence analysis indicated that the truncated m53BP1 protein expressed in m53BP1tr/tr animals failed to form foci in response to DNA damage as it was essentially absent from the nucleus (data not shown). This result is consistent with our transfection studies, which have shown that C-terminal determinants (ΔKINET) are necessary for focus formation. The relative growth of the mutant and the wild-type MEFs was reminiscent of what has been recently described for H2AX (24). To further characterize cells defective in m53BP1, we examined the cytological consequences of impaired m53BP1 function in early passage MEFs derived from m53BP1tr/tr and m53BP1+/+ animals. For this, exponentially growing MEFs (passage 2) were treated with 0, 0.5, or 1.5 Gy of γ-IR, and metaphase preparations were examined 2.5 h post-irradiation. Untreated MEFs derived from m53BP1tr/tr animals showed increased levels of chromatid gaps, breaks, and, to a lesser extent, exchanges when compared with those derived from m53BP1+/+ mice, suggesting an intrinsic genomic stability defect in the mutant cells (Fig. 5, B and C). More strikingly, irradiated MEFs derived from m53BP1tr/tr animals showed an ~2-fold increase in levels of chromatid breaks and gaps when compared with MEFs derived from wild-type mice (Fig. 5, B and C). Although MEFs from m53BP1tr/tr animals showed relatively high chromatid exchange rates at 0.5 Gy when compared with those from m53BP1+/+ animals, this difference was
less apparent at 1.5 Gy, perhaps due to the limited progression to mitosis of the most damaged cells from both populations during this time frame. One possible explanation for the increased frequencies of chromosomal aberrations observed in the \( m53BP1^{tr/tr} \) MEFs following irradiation might be a deficiency in a G2 checkpoint response whereby more damaged cells would still be permitted to enter mitosis and would be available for chromosome analysis. In fact, recent reports have implicated 53BP1 in the G2/M checkpoint (25–27). To examine this in our MEFs, either \( m53BP1^{tr/tr} \) or wild-type MEFs were treated with 0, 1.5, or 10 Gy of \( \gamma \)-IR, and cultures were analyzed for the fraction of cells showing phospho-histone H3 immunostaining (mitotic cells) either after 1 or 16 h post-irradiation (in the presence of colcemid). Although all cell types showed evidence of a partial G2/M block following irradiation, MEFs derived from \( m53BP1^{tr/tr} \) mice showed only a slight decrease, if any, in the G2 block when compared with MEFs derived from wild-type mice (data not shown). The minimal effects on the G2/M checkpoint observed in our \( m53BP1^{tr/tr} \) MEFs may be due to the nature of the truncated protein produced from the \( m53BP1^{tr/tr} \) allele that is expressed in our mutant animals described here.

53BP1 interacts with a variety of factors known to be involved in the maintenance of genomic stability including ATR, p53, H2AX, BRCA1, Chk2, and ATM (15, 20, 25, 27). The generation of murine animals defective in \( m53BP1 \) provides a valuable tool to further understand the role of the protein in the DNA damage response. The \( m53BP1^{tr/tr} \) allele expresses a truncated version of 53BP1, and this likely represents a

Fig. 4. Characterization of animals and cells defective in \( m53BP1^{tr/tr} \). A, survival of 4–6-week-old \( m53BP1^{tr/tr} \) and \( m53BP1^{+/-} \) mice after exposure to 7 Gy of \( \gamma \)-IR. Six animals from each genotype were used in the experiment. B, survival of 4–6-week-old \( m53BP1^{tr/tr} \) animals after exposure to 1.5 Gy of ionizing radiation.

Fig. 5. Chromosomal abnormalities in \( m53BP1^{tr/tr} \) cells. A, growth curve of MEFs derived from \( m53BP1^{tr/tr} \) (open diamonds) and \( m53BP1^{+/-} \) (closed circles). B, metaphase preparation of mutant MEF following 1.5 Gy of \( \gamma \)-IR. Note the presence of a chromatid gap, two chromatid breaks, and one chromatid exchange in the metaphase sample. C, relative frequencies of chromatid gaps, breaks, and exchanges in metaphases of wild-type and mutant MEFs following 0, 0.5, and 1.5 Gy of ionizing radiation.
significant impairment in some aspects of its function. m53BP1

is missing over 700 amino acids including the nuclear localization signal, the C-terminal BRCT motifs, and a kinetochore-binding domain. We have observed that this domain is also necessary for forming irradiation-induced nuclear foci. Indeed, the lack of detectable, irradiation-induced foci in mutant MEFs suggests that the protein cannot fully perform its functions as a DNA damage-response element. Moreover, the lethality observed for m53BP1

mice at higher doses of radiation (7 Gy) suggests that there are no other factors acting redundantly with m53BP1 with respect to this aspect of radiation resistance and indicates that m53BP1 is a critical element for double-stranded break repair. Therefore, the C-terminal 700 amino acids of m53BP1 encode important, functional determinants of the protein.

We observed that m53BP1

animals are growth-retarded as the males weigh, on average, 25% less than their wild-type littermates. The decreased thymus size, reduced T cell count, immature B cell population, and lack of progression out of DNIII for thymus T cells reveal that m53BP1

animals are immune-deficient. How m53BP1 contributes to this process remains to be established, but one possibility is that the protein participates in the maturation of T-cell receptors and immunoglobulins during V(D)J recombination, a process known to utilize DNA repair proteins (30). This is particularly interesting given the involvement of m53BP1 in double-stranded break repair as revealed by several factors including, most notably, the sensitivity of m53BP1

animals after exposure to 7 Gy of ionizing radiation. Indeed, sensitivity to ionizing radiation often correlates with impaired V(D)J joining (30). Moreover, H2AX defective-animals are also immune-deficient (24). As H2AX is required for the formation of 53BP1 foci and because it physically associates with 53BP1 (20), it is possible that an ordered pathway of assembly of DNA damage-response proteins at these programmed breaks may facilitate V(D)J recombination and maximize antibody diversity.

Our results show that genetic defects in m53BP1 result in a pleiotropic phenotype consistent with defects in DNA repair and checkpoint control. The phenotype of 53BP1-defective animals is quite similar to H2AX-deficient ones, consistent with the notion that H2AX operates upstream of 53BP1 in a DNA damage-response pathway. When such pathways are defective, cells cannot properly repair damaged DNA, a situation that may lead to increased genomic instability and the development of diseases such as cancer. For example, given the immune deficiencies in m53BP1

mice, one may anticipate the generation of lymphomas. In light of this, we have not observed the development of any cancerous phenotypes in our m53BP1-defective mice. Although there are a variety of possible reasons for this (i.e. genetic background, allele, etc.), it is interesting to note that mice nullizygous for H2AX also apparently fail to generate cancers.9 As H2AX and 53BP1 are not required for viability, it is possible that mutations in 53BP1, when combined with other mutations in critical DNA damage-response elements (i.e. H2AX, ATM, and p53) will lead to more severe defects in genomic stability, a process that may then lead to the development of cancer. The analysis of cells derived from these crosses is likely to provide more insight into how 53BP1 functions in the DNA damage response in concert with its various interacting partners.

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