Impaired Skin and Mammary Gland Development and Increased γ-Irradiation–Induced Tumorigenesis in Mice Carrying a Mutation of S1152-ATM Phosphorylation Site in Brca1

Sang Soo Kim,1,2 Liu Cao,2 Hye Jung Baek,1 Sung-Chul Lim,3 Cuiling Li,2 Rui-Hong Wang,2 Xiaoling Xu,2 Kwan Ho Cho,1 and Chu-Xia Deng2

1Radiation Medicine Branch, National Cancer Center, Goyang, Korea; 2Genetics of Development and Disease Branch, National Institute of Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, Maryland; and 3Department of Pathology, College of Medicine, Chosun University, Gwangju, Korea

Abstract

The tumor suppressor BRCA1 interacts with many proteins and undergoes multiple modifications on DNA damage. ATM, a key molecule of the DNA damage response, phosphorylates S1189 of BRCA1 after γ-irradiation. S1189 of BRCA1 is known as a unique ATM phosphorylation site in BRCA1 exon 11. To study the functions of ATM-dependent phosphorylation of BRCA1-S1189, we generated a mouse model carrying a mutation of S1152A (S1152 in mouse Brca1 corresponds to S1189 of BRCA1-S1189, we generated a mouse model carrying a mutation of S1152A (S1152 in mouse Brca1 corresponds to S1189 in human BRCA1) by gene targeting. Brca11/1152A/1152A mice were born at the expected ratio, unlike that seen in previous studies of Brca1-null mice. However, 36% of Brca11/1152A/1152A mice exhibited aging-like phenotypes including growth retardation, skin abnormalities, and delay of the mammary gland morphogenesis, with an increase in apoptosis. Mutant mice were hypersensitive to high doses of γ-irradiation, displaying shortened life span and reduction in intestinal villus size, associated with increased apoptosis. Aging-unaffected 18-month-old Brca11/1152A/1152A female mice also showed mammary gland abnormalities with increased levels of cyclin D1 and phospho-ER-α, such as Brca1-Δ11 mutation. On low-dose γ-irradiation, they suffered a marked increase in tumor formation with an abnormal coat pattern. Furthermore, Brca11/1152A/1152A embryonic fibroblasts failed to accumulate p53 on γ-irradiation with delayed phosphorylation of p53-S23. These observations indicate that ATM-mediated phosphorylation of S1189 is required for BRCA1 functions in the modulation of DNA damage response and in the suppression of tumor formation by regulating p53 and apoptosis. [Cancer Res 2009;69(24):9291–300]

Introduction

Ataxia-telangiectasia is a human autosomal recessive disorder characterized by progressive neurodegeneration, immunodeficiency, and cancer predisposition (1). The ataxia-telangiectasia cellular phenotype includes chromosomal instability, radiosensitivity, and failure to adequately activate cell cycle checkpoints (2). Ataxia-telangiectasia-mutated (ATM), the gene product defective in ataxia-telangiectasia, is a member of the phosphoinositide 3-kinase family, which is involved in the recognition of damage in DNA (3).

Germ-line mutations in BRCA1 and BRCA2 are responsible for the majority of hereditary breast cancers and cause almost all familial cases involving both breast and ovarian cancers (4, 5). The BRCA1 protein is a tumor suppressor that has a crucial role in the maintenance of genomic integrity. BRCA1 achieves this by integrating important cellular processes such as regulation of genetic stability, DNA damage repair, centrosome duplication, apoptosis, and cell cycle control (6). BRCA1 contains 24 exons that encode proteins of 1,863 and 1,812 amino acids in the human and mouse, respectively (7, 8). Notably, more than 60% of the protein is encoded by an unusually large exon, exon 11, which is 3.4 kb in length. In addition to the full-length BRCA1 protein (BRCA1-FL), a deletant, BRCA1-Δ11, arises from in-frame splicing between exon 10 and exon 12, but retains the highly conserved NH2-terminal RING finger and COOH-terminal BRCT domains found in full-length BRCA1 (9–12). To study the functions of BRCA1 and to create animal models for BRCA1-associated breast cancer, mice carrying various mutations in Brca1 have been generated by gene targeting (reviewed in ref. 13). Brca1-null embryos die at embryonic day (E) 6.5 to 8.5 (14–16), whereas embryos that lack Brca1-FL due to targeted deletion of exon 11 (Brca11/11) but still express the Brca1-Δ11 isoform die at E12 to E18.5 (17). In addition, surviving Brca11/11 mice that had lost one or both p53 alleles exhibited aging phenotypes with tumor formation (17, 18). Moreover, Cre-mediated excision of exon 11 of Brca1 in mouse mammary epithelial cells caused abnormal ductal development attributable to induction of apoptosis (19). However, Brca11/11 mutant mice, which lack the Brca1-Δ11 isoform, did not display any developmental defects, and the incidence of tumor formation in such animals was significantly lower than that in Brca11/11 mice, indicating that exon 11 of Brca1 is essential for proper functioning of Brca1 in development and tumor suppression (20).

The DNA damage response involves the sensing of DNA damage followed by transduction of the damage signal to a network of cellular pathways, including cell cycle checkpoints, DNA repair, and the apoptotic system (21). It has been shown that BRCA1 undergoes regulation by phosphorylation on DNA damage and cell cycle progression (22). In this network, ATM is also a critical regulator of checkpoint signal cascades; it phosphorylates and activates several molecules including H2AX, p53, and CHK1 to execute the DNA damage response (2, 23). ATM also phosphorylates BRCA1 in response to ionizing radiation in vivo and in vitro in a region that contains clusters of serine and glutamine residues (24). Analysis
by mass spectrometry showed that seven serine residues (S1189, S1330, S1423, S1457, S1466, S1524, and S1542) of BRCA1 were phosphorylated. ATM has also been reported to interact with BRCA1 as part of a large multisubunit protein complex of tumor suppressors, termed BASC (25). These results indicate that communication between BRCA1 and ATM is critical for an appropriate response to DNA damage and may provide a molecular explanation for the role of BRCA1 and ATM in preventing tumor formation.

However, the functional role of phosphorylation by ATM of BRCA1 in the progression of breast cancer remains elusive. To address the potential function of BRCA1 exon 11, we mutated S1152 of mouse Brca1, which is equivalent to S1189 of human BRCA1, a unique ATM phosphorylation site in exon 11, and studied the biological consequences using mutant mice and cultured mutant cells. Our data indicate that ATM-mediated phosphorylation of S1152 mediates some Brca1 functions in the modulation of homeostasis, longevity, and genetic integrity, and that disruption of this site results in increased tumor formation after DNA damage in mutant mice.

Materials and Methods

Mouse treatment and analysis. Mice were monitored at least twice a week for possible symptoms related to illness and tumor formation.

![Figure 1. Aging-like phenotypes of Brca1<sup>S1152A/S1152A</sup> mice.](https://cancerres.aacrjournals.org) A, life span and skin abnormalities of Brca1<sup>S1152A/S1152A</sup> mice. A representative photograph of skin damage in aging-affected Brca1<sup>S1152A/S1152A</sup> animal is shown with WT mouse. Arrows, area of wounds. Right, magnified images of the boxed areas. B, Brca1<sup>S1152A/S1152A</sup> female mice weighed significantly less than their WT littermates between 7 and 11 mo of age (n = 10; P < 0.01). C, pattern of hair regrowth and H&E staining of skin sections 1 wk after shaving and wounding. Right, transverse (1st and 2nd image) and tangential (3rd image) sections of the skins. D, quantitative measurement of skin and hypodermis thickness. Skin tissues were collected in the center of back skin from WT (n = 8) and Brca1<sup>S1152A/S1152A</sup> mutant (n = 7) mice. After histological processing, the thicknesses of the skins were measured in three different locations in each section under a microscope. *, P < 0.01. Bar, 200 μm. Ep, epidermis; Sc, subcutaneous; S1152A, Brca1<sup>S1152A/S1152A</sup>.
Animals were also subjected to γ-irradiation to check sensitivity toward tumorigenesis after DNA damage. In the high-dose γ-irradiation study, mice were given a single irradiation of 8 Gy at 8 wk of age. For the low-dose γ-irradiation study, female mice at 8 wk of age were irradiated at 3 Gy four times at 1-wk intervals (26). When mice developed symptoms and tumors, tissues were collected, divided, frozen in liquid nitrogen, and stored at −80°C or fixed in 10% (v/v) buffered formalin and embedded in paraffin for staining. We also carried out whole-mount staining of mammary glands.

Mouse embryonic fibroblasts and analysis. Mouse embryonic fibroblasts (MEF) were derived from E14.5 embryos generated from intercrosses as previously described (19).

Immunohistochemical staining of skin sections was done on WT mice and aging-affected (A) and 18-mo-old (B) Brca1+/S1152A/S1152A mice. Sections were imaged using primary antibodies against Ki67, K6, and AE13. Bar, 200 μm.

Results

Targeted disruption of the ATM phosphorylation site, S1152, in mouse Brca1. In response to ionizing radiation, ATM phosphorylates BRCA1 at seven serine residues (S1189, S1330, S1423, S1457, S1466, S1524, and S1542; ref. 24). Among these, S1189 is the sole residue conserved in mouse Brca1 and is located in exon 11. Thus, we substituted S1152 of mouse Brca1 with A1152, using a cotransfer-type targeting construct (Supplementary Fig. S1A). Southern blot analysis detected homologous recombination at the Brca1 locus in 10 of 82 G418/FIAU doubly resistant embryonic stem cell (ES) clones (Supplementary Fig. S1B and C). After confirming that the mutant cells contained the S1152A mutation by Apal digestion and sequencing (data not shown), three correctly targeted ES clones were injected into blastocysts, and germ-line transmission was obtained from all three. Mice carrying the targeted disruption were further crossed with Ella-Cre transgenic mice that express Cre in the germ line (27) to delete the plaxPnoe gene (Supplementary Fig. S1A). Mice carrying a heterozygous mutation of Brca1-S1152A (Brca1+/S1152A) were phenotypically normal and were further crossed to produce Brca1−/S1152A/S1152A animals (Supplementary Fig. S1D). Our data indicated that Brca1−/S1152A/S1152A mice were present in a Mendelian ratio at weaning (data not shown). Western blot analysis indicated that Brca1-S1152A was expressed in mutant cells at levels similar to those of WT protein (Supplementary Fig. S1E).

Our analysis indicated that both male and female mice with homozygous mutations of Brca1-S1152A showed no fertility abnormality. This observation indicated that the Brca1-S1152A mutation caused no obvious developmental defects.

Figure 2. Immunohistochemical analysis of Brca1−/S1152A/S1152A mouse skin. Immunohistochemical staining of skin sections was done on WT mice and aging-affected (A) and 18-mo-old (B) Brca1−/S1152A/S1152A mice. Sections were imaged using primary antibodies against Ki67, K6, and AE13. Bar, 200 μm.
Brca1S1152A/S1152A mice exhibit aging-like phenotypes. The Brca1S1152A/S1152A mutant mice survived to adulthood, enabling us to investigate the effect of ATM phosphorylation of Brca1 exon 11 by monitoring phenotypes. We followed a group of mutant mice (n = 78) and found that 30 displayed abnormalities within the first year of life (Fig. 1A). Twenty-eight of 30 mice were small, thin, and hyperactive, with severe dermatitis (Fig. 1A, arrow) in the dorsal region, from neck to tail. Two mice died of thymic lymphoma. Simultaneously, we compared the weights of Brca1S1152A/S1152A mice and WT animals at 1-month intervals from 2 to 11 months (Fig. 1B). The weight of Brca1S1152A/S1152A mice (n = 15) did not increase after 6 months and the differences between WT and mutant animals were statistically significant after month 7 (P < 0.01).

To further examine whether the dermatitis resulted from a defect in wound healing, we investigated the healing pattern after skin incision in Brca1S1152A/S1152A mice compared with WT animals. As shown in Fig. 1C, wounded mutant skins healed but displayed either complete failure or delayed recovery of hair regrowth after shaving (arrows in Fig. 1C). When skin areas were analyzed by H&E staining, stained skin of WT mice revealed a typical architecture of...
the epidermal/dermal junction, with many hair follicles. In contrast, skins of mutant mice clearly showed an abnormal pattern of hair regeneration with shrinkage of the subcutaneous layer and a lower density of hair follicles in the epidermal layer (Fig. 1C and D).

To identify the cause of skin abnormalities in Brca1-S1152A mice, we used immunohistologic analysis to study skin sections from aging-affected Brca1S1152A/S1152A animals in comparison with WT mice. In proliferating cells of WT mice, a Ki67-positive population was localized at the hair bulb region, whereas such cells were distributed in many tiny follicles in Brca1S1152A/S1152A animals. To determine whether the Brca1-S1152A mutation corrupted skin architecture, we assessed skin sections using molecular markers of hair follicles (Fig. 2A). Thus, we examined the expression of K6 and AE13, which are characteristic of keratinocytes of the outer root sheath and inner root sheath, respectively, in WT animals. In contrast, hair follicles of Brca1-S1152A mice displayed colocalization of K6 and AE13, unlike the separated WT phenotype. Interestingly, the hair follicle pattern in mutant mice was similar to that of 18-month-old animals in distribution and location of molecular markers including Ki67 and K6 (Fig. 2B). When the expression pattern of hair follicle markers in aged populations was compared, mutant animals displayed reduced expression of Ki67, K6, and AE13 compared with age-matched, WT mice. Together, these data indicate that the ATM-modifiable S1152 residue of Brca1 is required for proper skin structure formation and hair regeneration, and that loss of ATM regulation of Brca1-S1152 resulted in shortening of life span with skin abnormalities characteristic of premature aging.

Delayed development and increased apoptosis in Brca1-S1152A mutant glands. Development of the mammary gland proceeds in distinct stages. Ductal elongation and branching occur mainly during puberty, whereas alveolar proliferation and differentiation take place during pregnancy (28). Deletion of Brca1 from mammary epithelial cells resulted in aberrant mammary development (19). This finding prompted us to investigate whether Brca1-S1152A mutation caused phenotypical abnormalities in the development of the mammary gland.

To observe the ductal and alveolar development of the gland, we performed whole-mount staining of abdominal mammary glands of 2-month-old virgin female mice (Fig. 3A). We observed
substantial alveolar development in WT animals (Fig. 3A, left). In contrast, the mammary gland of mutant mice was completely filled with a fat pad but displayed a delay in development with respect to duct and lobular formation. In Brca1-S1152A mutant mice, 22% (6 of 27) and 33% (9 of 27) exhibited incomplete ductal development or lobular formation, respectively (Fig. 3A, right). H&E staining of mammary gland sections clearly showed the aberrant structure of the mutant gland compared with WT (Fig. 3B). However, despite the occurrence of mammary gland abnormalities in adolescent mutant mice, mutant animals showed a mature structure of mammary gland at 4 months of age (data not shown). It is because the structure of normal mammary gland accomplishes within 2 to 3 months in virgin mice, and this structure remains the same unless the mice become pregnant. Apparently, the Brca1-S1152A mutation delays, but does not block, the process of mammary gland development.

We have previously shown that pregnancy induced the hyperplastic changes in the mice lacking Brca1 in mammary gland (19). Thus, we also examined whether Brca1-S1152A mutant mice exhibited the mammary gland abnormalities in pregnancy, lactation, and involution periods. However, we could not find any differences between WT and mutant mice in whole-mount staining and histologic analysis (data not shown). We also compared the capability of nursing the offspring and did not find any difference between Brca1+/S1152A and Brca1S1152A/S1152A mutant mice. The average numbers of offspring of Brca1+/S1152 (65 litters) and Brca1S1152A/S1152A (24 litters) at weaning time were 7.8 and 7.1 pups, respectively, with no obvious difference in body weight.

It has been reported that loss of Brca1 in the mammary gland causes increased apoptosis of epithelial cells and results in developmental abnormality with blunted ductal morphogenesis (19). To study the mechanisms underlying the restricted development of the Brca1-S1152A mammary gland, we assessed cell proliferation and apoptosis in the thoracic mammary glands of these mice (Fig. 3C and D). We observed similar levels of BrdUrd positivity (26.8% versus 23.9%), but a statistically significant difference in apoptosis was apparent in Brca1-S1152A mutant mice (3.3%) compared with WT mice (0.19%). The results therefore indicate that signal communication between Brca1 and ATM is required for proper development of the mammary gland in terms of regulation of apoptosis.

**Brca1-S1152A mutant mice display hypersensitivity to γ-irradiation.** Several lines of evidence indicate that a deficiency in BRCA1 causes a marked increase in DNA damage on γ-irradiation (6). To determine whether the Brca1-S1152A mutation

Figure 5. Mammary gland abnormalities in Brca1S1152A/S1152A mice. Whole-mount (A), H&E (B), and immunohistochemical (C and D) staining of mammary glands from Brca1S1152A/S1152A and WT mice at 18 mo of age. Right, magnified images of the boxed areas. Immunohistochemical imaging was achieved using primary antibodies against cyclin D1 (C) and phospho-ER-α (D). Bar, 100 μm.
influenced sensitivity to \(\gamma\)-irradiation, we examined the levels of p53 protein in MEFs derived from \(Brca1^{S1152A/S1152A}\) and control embryos after 10-Gy irradiation. In WT MEFs, p53 levels began to increase from 1 hour after irradiation and reached maximal levels by 4 hours. However, in \(Brca1^{S1152A/S1152A}\) MEFs, the level of p53 in untreated was rather high, but it did not increase after \(\gamma\)-irradiation (Fig. 4A). In addition, the level of p53 phosphorylated at Ser\(^{23}\) (p53-S23) rapidly increased in WT MEFs, by about 3.3-fold 1 hour after irradiation, and decreased substantially by 2 hours. In contrast, the level of p53-S23 peaked at 2 hours (1.6-fold induction) and decreased notably by 4 hours after treatment of \(Brca1^{S1152A}\) MEFs (Fig. 4A). We next investigated whether the \(Brca1-S1152A\) mutation modified cell cycle checkpoints in MEFs after \(\gamma\)-irradiation. However, we found that \(Brca1^{S1152A/S1152A}\) MEFs showed no statistically significant defect in any of several cell cycle checkpoints analyzed, including the intra-S, G2-M, and G1-S checkpoints, after treatment with 10-Gy irradiation (data not shown).

To further investigate the effect of ATM phosphorylation of Brca1 exon 11, we irradiated \(Brca1^{S1152A/S1152A}\) mice and WT animals and compared their responses. Surprisingly, irradiation with 8 Gy markedly decreased the life span of \(Brca1-S1152A\) mutant mice (mean, 11.3 days) compared with WT animals (mean, 18.6 days; Fig. 4B). No mutant \(Brca1^{S1152A/S1152A}\) mice survived for longer than 14 days, whereas 7 of 14 (50%) WT animals lived beyond that time.

In an earlier study, animals carrying a null mutation in ATM displayed radiosensitivity of the intestine with extensive structural changes seen on irradiation (29). Therefore, we were interested in determining whether \(Brca1-S1152A\) mice exhibited a similar phenotype. Thus, WT and \(Brca1^{S1152A/S1152A}\) mice were irradiated at 8 Gy, followed by histologic analysis of intestinal samples taken 3 days after irradiation. As shown in Fig. 4C, a marked shortening and rounding of villi was observed in \(Brca1-S1152A\) mutant mice but not in WT animals. The villus-to-crypt height ratios in the small intestine were 4 ± 0.6 and 1.8 ± 0.4 in WT and \(Brca1-S1152A\) mutant mice, respectively. Moreover, apoptotic signals in \(Brca1^{S1152A/S1152A}\) mice were detected in a broad region of atrophic small intestinal mucosa, whereas such signals were seldom seen in the mucosa of WT animals (Fig. 4D).

The results indicate that ATM and BRCA1 cooperate to prevent radiation-induced apoptosis and that BRCA1-S1189 is a key residue.

**Figure 6.** Reduced survival and high tumor incidence in \(Brca1^{S1152A/S1152A}\) mice treated with \(\gamma\)-irradiation. A, Kaplan-Meier survival curve of WT (\(n = 22\)) and \(Brca1^{S1152A/S1152A}\) (\(n = 16\)) mice after irradiation. B, tumor spectrum of mice after irradiation. Two mutant mice had tumors in more than one organ. C, WT and \(Brca1^{S1152A/S1152A}\) mice at 10 mo after irradiation. D, immunohistochemical staining of mammary tumor sections from \(Brca1^{S1152A/S1152A}\) and WT mice. Images of sections were detected using primary antibodies against cyclin D1, ER-\(\alpha\), phospho-ER-\(\alpha\) (p-ER-\(\alpha\)), and p53. Bar, 50 \(\mu\)m.
in this collaboration. Loss of this residue inhibits the DNA damage response induced by γ-irradiation.

**Mammary gland hyperplasia and accelerated γ-irradiation-induced tumorigenesis in Brca1Δ11p53−/− mice.** Our previous studies revealed that Brca1Δ11p53−/− mice showed normal mammary gland development, and most of them developed mammary tumors within 6 to 10 months, whereas none of the p53−/− mice exhibited the neoplastic abnormalities in mammary gland in the same period (17). However, Brca1Δ11p53−/− mice did not reveal any spontaneous mammary tumor formation (15 animals). However, in six female Brca1Δ11p53−/− mice examined at 18 months of age, very dense branches were seen in mammary glands, with small hyperplastic foci, whereas no WT animal studied showed similar abnormalities at the same age (Fig. 5A). Pathologic analysis of abnormal mammary glands from Brca1Δ11p53−/− mice displayed atypical hyperplasia (Fig. 5B). The S1152A-1 and S1152A-2 mutants showed lobular carcinomas in situ and atypical lobular hyperplasia. In the S1152A-3 mutant, we found the pathologic findings described above and, additionally, an atypical microglandular adenosis. These abnormalities are not indicative of breast cancer but are accepted to be important markers for development of aggressive cancer (30).

To examine the molecular characteristics of hyperplastic mammary glands from Brca1Δ11p53−/− mice, we compared the expression levels of cyclin D1 and phosphorylated ER-α in sections from Brca1Δ11p53−/− mice with those in normal mammary glands of age-matched WT animals. As shown in Fig. 5C and D, mammary epithelial cells of Brca1Δ11p53−/− mice expressed greater levels of cyclin D1 and activation of ER-α than did WT animals. This indicates that the mutation in Brca1Δ11p53−/− increased the expression of cyclin D1 and phosphorylation of ER-α, both of which are associated with increased cell proliferation.

Next, to determine the significance of ATM phosphorylation of the S1152 residue of BRCA1 in tumor prevention, we treated mice with a sublethal dose of γ-irradiation (3 Gy) four times at 1-week interval and studied the response. A significant tumorigenic alteration was induced by γ-irradiation of mutant mice, and this commenced at 3 months of age. Within the first year of life, 80% of mutant mice had developed tumors and all animals exhibited tumors by 18 months of age (Fig. 6A). In contrast, less than 20% (3 of 16) WT mice developed tumors during the same period. The main type of tumor was lymphoma in both mutant and WT animals. However, mutant mice showed a significantly higher frequency of tumor development than did WT animals. Four mutant mice developed mammary tumors, three developed liver tumors, and two exhibited tumors in two different organs (Fig. 6B). In addition to tumor formation, Brca1Δ11p53−/− mice showed hair graying after γ-irradiation (Fig. 6C). The cause and consequences of gray hair development in Brca1Δ11p53−/− mice after irradiation are not clear. However, gray hair development has also been reported in ATM- and Per2-deficient mice and in Brca1Δ971AΔ971A animals, which also showed a high incidence of tumor formation (26, 31, 32).

Next, we performed an immunohistochemical analysis of mammary tumors from Brca1Δ11p53−/− mice to determine whether the pattern of molecular markers was similar to that in Brca1Δ11/Δ11 mice (Fig. 6D). Differentiated adenocarcinomas from Brca1Δ11p53−/− mice were highly positive for cyclin D1, ER-α, and phospho-ER-α but were negative for p53. Interestingly, neither ER-α nor phospho-ER-α was expressed in less differentiated adenocarcinomas. These results are in agreement with our previous data illustrating a gradual reduction in ER-α expression with tumor progression in mammary tumors of Brca1Δ11/Δ11p53−/− mice (33).

Together, these results show that ATM phosphorylation of the S1189 residue of BRCA1 is required for tumor prevention through modulation of p53 activity and apoptosis. The absence of this regulatory pathway leads to carcinogenesis.

**Discussion**

The regulation of cellular responses to DNA damage is indispensable for maintenance of genomic stability. BRCA1 has a critical role in the early stages of this pathway through interaction with ATM, CHK2, and p53 (24, 34, 35). Our previous studies of Brca1 knockout mice have shown the complicated mechanism by which BRCA1 protein affects genetic integrity. Comparisons between mutant and WT animals may provide clues to the understanding of BRCA1 biological functions. Null mutations in mouse Brca1 led to embryonic lethality at E6.5 to E8.5 (14–16). However, most Brca1Δ11/Δ11 mice lacking the full-length protein but expressing the Δ11 isoform died at E12.5 to E18.5, and a small proportion of mutant mice survived to adulthood but showed significant aging phenotypes (17, 18). Brca1Δ11/Δ11 mice survived to adulthood in a mutated background for p53, Chk2, p53BP1, or ATM, and all double mutant mice exhibited a high tumor incidence and phenotypes related to aging (18, 35, 36). Brca1Δ11/Δ11 cells grew poorly and were defective in both the G2-M cell cycle checkpoint and the spindle checkpoint (11, 37). In contrast, Brca1Δ11/Δ11 mice lacked the Δ11 isoform, but with the full-length protein, did not display any phenotype related to premature aging (20). Moreover, Brca1Δ11/Δ11 cells grew normally and did not show any obvious defects in several major cell cycle checkpoints analyzed, indicating that exon 11 of BRCA1 may have an essential role in these processes.

During the DNA damage response, BRCA1 is extensively phosphorylated by several protein kinases, and this process is tightly linked to biological consequences (22). Thus, we tested whether impairment of regulation by phosphorylation of exon 11 of BRCA1 was responsible for the phenotypes characteristic of Brca1Δ11/Δ11. Previously, it had been reported that phosphorylation by CHK2 of S988 of BRCA1, which is located in exon 11, was followed by a change in BRCA1 intracellular location (38). Thus, we generated and investigated a mouse model carrying the mutation S971A (S971 in mouse BRCA1 corresponds to S988 of human BRCA1; ref. 26). We showed that Brca1Δ971AΔ971A mice were at a moderately increased risk of spontaneous tumor formation, with a majority of females developing uterus hyperplasia and ovarian abnormalities by 2 years of age. In addition, these mice exhibited acceleration of tumor formation after irradiation treatment. However, Brca1Δ971AΔ971A animals did not display any developmental defect, irradiation sensitivity, or premature aging; these phenotypes were characteristic of Brca1Δ11 mutant animals.

In the present report, we showed that Brca1Δ11p53−/− mutant and Brca1Δ11 mutant mice shared phenotypes that were not found in Brca1Δ971AΔ971A mutant animals. First, Brca1Δ11p53−/− mice exhibited delayed development of mammary ducts and lobules, accompanied by an increased level of apoptosis, as also shown in conditional Brca1-mutant mice, indicating that disruption of Brca1 causes a developmental defect in the mammary gland by triggering apoptosis (19). Second, the absence of the Brca1 full-length isoform causes senescence in mutant embryos and cultured cells, as well as aging and tumorigenesis in adult mice (18, 35). The haploid loss of p53 or ATM overcame embryonic senescence but failed to prevent...
the adult mutant mice from aging prematurely, as shown by a decreased life span, reduced body fat deposition, osteoporosis, skin atrophy, and poor wound healing. Similarly, \textit{Brca1}\textsuperscript{Δ11/S1152A} mice displayed aging-like phenotypes including shortened life span, reduced body weight, frequent skin damage, and lack of skin fat deposition. Next, we showed that the molecular markers of mammary tumors from \textit{Brca1-S1152A} mutant mice exhibited a pattern similar to that in \textit{Brca1-Δ11} mutant animals. Cyclin D1 was detected in both types of mutant mice in several stages of abnormal mammary gland development (39). ER-α signals were initially positive but became negative on tumor progression, as also noted in the triple-negative tumors seen in \textit{BRCA1}-deficient humans (33). Lastly, our previous report showed that \textit{Brca1}-mutant embryos exhibited a marked reduction in growth after \textit{γ}-irradiation (16). In addition, \textit{Brca1-S1152A} mutant mice showed reduced survival, gut hypersensitivity, and increased tumor incidence after \textit{γ}-irradiation. Although there are similarities in the phenotypes of \textit{Brca1-Δ11} and \textit{Brca1-S1152A} mutant mice, abnormalities in animals carrying the \textit{Brca1-Δ11} mutation were much more severe than those in mice with the \textit{Brca1-S1152A} mutation. This is because exon 11 of \textit{BRCA1} comprises more than half the coding region and interacts with several proteins including Rad50, Rad51, and Brca2 (40). Thus, loss of Brca1 exon 11 could logically result in the development of more severe phenotypic characteristics that are associated with a single-point mutation in an ATM phosphorylation site in this region. However, only \textit{Brca1-S1152A} mutant mice showed similar phenotypes to those of \textit{Brca1-Δ11} mutant mice; \textit{Brca1-S971A} and \textit{Brca1-FL} mutant animals did not. Interestingly, \textit{Brca1}\textsuperscript{Δ11/S1152A} mice resembled ATM null-mutant animals, which also displayed premature aging, \textit{γ}-irradiation hypersensitivity, and induction of tumorogenesis (29, 31). Together, our data indicate that disruption of \textit{BRCA1} S1189 phosphorylation by ATM impairs the ability of \textit{BRCA1} and/or to inhibit tumor prevention and premature aging by inhibiting cross talk between these two proteins.

In summary, \textit{BRCA1} is a large protein with multiple functional domains that interact with many proteins (40). In our continuous efforts to address the biological functions of \textit{BRCA1}, we specifically mutated S1152 of mouse \textit{Brca1}, which is uniquely phosphorylated by ATM in \textit{BRCA1} exon 11. Our data indicate that S1152 of \textit{BRCA1} has a specific role in longevity and that alteration of this amino acid results in the development of aging-like phenotypes including growth retardation and skin abnormalities. Our data also show that alteration of S1152 in \textit{BRCA1} results in a change in the response to DNA damage by affecting regulation of p53 activation and apoptosis. Although the influence of S1152-\textit{BRCA1} on tumor prevention is greater than that in mice with a large deletion of the coding region, \textit{Brca1}\textsuperscript{Δ11/S1152A} mutant animals exhibited atypical hyperplasia in the mammary gland over a long period. The mutant mice were also highly susceptible to tumorogenesis after \textit{γ}-irradiation treatment, with increased tumor incidence and latency. These observations indicate that ATM-mediated phosphorylation of S1189 is required for \textit{BRCA1} functions in the modulation of DNA damage response and in the repression of tumor formation.

\textbf{Disclosure of Potential Conflicts of Interest}

No potential conflicts of interest were disclosed.

\textbf{Acknowledgments}

Received 7/1/09; revised 10/27/09; accepted 10/27/09; published OnlineFirst 12/8/09.

\textbf{Grant Support:} Intramural Research Program of the National Institute of Diabetes, Digestive, and Kidney Diseases, NIH (Bethesda, MD) to C.-X. Deng, and the National Cancer Center of Korea (NCC-091020), the Korea Healthcare Technology R&D Project (A090095), and the National Research Foundation of Korea (2009-0070609) to S.S. Kim.

\textbf{The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.}

\textbf{References}


Impaired Skin and Mammary Gland Development and Increased \( \gamma \)-Irradiation–Induced Tumorigenesis in Mice Carrying a Mutation of S1152-ATM Phosphorylation Site in Brca1

Sang Soo Kim, Liu Cao, Hye Jung Baek, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-09-2418

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2009/12/18/0008-5472.CAN-09-2418.DC1

Cited articles
This article cites 40 articles, 16 of which you can access for free at:
http://cancerres.aacrjournals.org/content/69/24/9291.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/69/24/9291.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link:
http://cancerres.aacrjournals.org/content/69/24/9291.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.