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Mutant *Brca2/p53* mice exhibit altered radiation responses in the developing mammary gland

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Abstract

Appropriate balance between proliferation and apoptosis is critical for mammary gland development and is often altered during tumorigenesis. Carcinogens like radiation induce DNA damage and activate protective responses such as cell cycle arrest and apoptosis. We used mice carrying *Brca2*$^{-/-}$ and/or *p53*$^{-/-}$ mutations to evaluate the individual and combined effects of these genes on cell proliferation and apoptosis in the developing mammary gland. Mice were exposed to 5 Gy of radiation or chamber exposure (controls) followed by injection with BrdU. Mammary glands were collected 6 h post-radiation exposure and evaluated for proliferation (BrdU) and apoptosis (TUNEL) in terminal end buds (TEB) and ducts. Under control conditions, the *Brca2* mutation reduced proliferation and apoptosis in TEB but not ducts, whereas the *p53* mutation reduced apoptosis in TEB and ducts but did not influence proliferation. Despite these alterations in proliferation and/or apoptosis, neither mutation, either individually or combined, significantly altered the overall balance between the two as measured by the proliferation to apoptosis ratio (growth index). Following irradiation, the *Brca2* mutation had no significant effect on proliferation or apoptosis, whereas the *p53* mutation resulted in reduced apoptosis in TEB and ducts but did not significantly influence proliferation. Neither mutation by itself altered the growth index in the TEB after irradiation although combined *Brca2/p53* mutation caused significantly increased proliferation, reduced apoptosis, and an elevated growth index in TEB and ducts. These results reveal both independent and collaborative growth regulatory roles for *Brca2* and *p53* under normal and adverse environmental conditions. Additionally, we demonstrate the importance of gene–environment interactions by showing

Abbreviations: WT; wild-type (*Brca2*$^{+/+}$*p53*$^{+/+}$); p53$^{-/-}$, *p53*-mutant; *Brca2*$^{-/-}$, *Brca2*-mutant; dKO, double-mutant (*Brca2*$^{-/-}$*p53*$^{-/-}$); TEB, terminal end bud(s); BrdU, 5-bromo-2-deoxyuridine; DMBA, 7, 12-dimethylbenz[*a*]anthracene; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; LI, labeling index; GI, Growth index, proliferation:apoptosis ratio

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that *Brca2*- and *p53*-deficient mice can compensate for their genetic deficiencies under control conditions but not after exposure to radiation. We also demonstrate distinct spatial differences in the cellular functions of *Brca2* and *p53* and show that combined mutation of both genes is more detrimental than loss of either gene alone.
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Keywords: Brca2; p53; Apoptosis; Proliferation; Radiation

Introduction

Germline mutations in *BRCA2* confer an increased risk of breast cancer in both women and men (Easton et al., 1997). Several lines of evidence suggest that loss of p53 function may be an important event in the genesis of *BRCA2*-associated cancers, although the mechanism of how this occurs is not known (Crook et al., 1998; Greenblatt et al., 2001; Gretarsdottir et al., 1998; Jonkers et al., 2001; Medina et al., 2002; Ramus et al., 1999; Tong et al., 2000). Brca2 and p53 physically and functionally interact (Marmorstein et al., 1998) and recent studies suggest their relationship is antagonistic. p53 has an inhibitory effect on the *Brca2* promoter and is capable of inhibiting homologous recombination, a process mediated by Brca2 (Mekeel et al., 1997; Sturzbecher et al., 1996; Wu et al., 2003). Brca2 can negatively regulate p53-mediated transcriptional activity (Marmorstein et al., 1998), whereas deficiency of Brca2 can result in elevated p53 expression (Connor et al., 1997; Patel et al., 1998). Loss of either gene results in genomic instability and an impaired ability to respond to radiation-induced DNA damage (Bertrand et al., 1997; Connor et al., 1997; Foray et al., 1999; Sharan et al., 1997; Tutt et al., 1999, 2002).

Ionizing radiation activates a network of signaling pathways involved in cell cycle arrest, apoptosis, and DNA repair (Li et al., 2001). p53 has a role in all three of these processes (Lowe et al., 1993; MacCallum et al., 1996; Prives and Hall, 1999), although the function of Brca2, beyond its role in DNA repair, is much less certain and sometimes conflicting. For example, one study revealed decreased apoptosis in thymocytes from *Brca2*-mutant mice exposed to the DNA damaging agent etoposide (Flores et al., 2002), whereas another study reported increased rates of spontaneous apoptosis in *Brca2*-null T-lymphocytes (Cheung et al., 2002). In contrast, Patel et al. (1998) determined that apoptotic mechanisms were largely unaffected in *Brca2*-deficient lymphoid cells and similar observations have been made with *Brca2*-deficient embryos (Suzuki et al., 1997). Studies utilizing the hamster cell line V-C8, which carries mutant *Brca2*, revealed increased levels of apoptosis in response to the DNA damaging agent mitomycin C (Papouli et al., 2000). Similarly, Yan et al. (2004) showed that a mouse derived *Brca2*-heterozygous cell line and Capan-1 cells, which carry a mutant *BRCA2* gene, are more sensitive to apoptosis when exposed to 7, 12-dimethylbenz[*a*]anthracene (DMBA).

A potential role for Brca2 in cell cycle regulation has been suggested, although it is often difficult to differentiate direct Brca2-mediated effects from secondary effects related to its role in DNA repair. Studies showing increased Brca2 expression in rapidly proliferating cells and those that describe Brca2's cell cycle specific expression provide support for involvement in cell cycle regulation (Rajan et al., 1996; Vaughn et al., 1996). Early studies with *Brca2*-deficient embryos showed a marked proliferation defect that was less severe on a *p53*-null background (Friedman et al., 1998; Ludwig et al., 1997). *Brca2*-deficient mouse embryonic fibroblasts revealed a similar proliferation defect characterized by cell cycle arrest in G1 and G2/M and increased expression of p53 and p21 (Connor et al., 1997; Patel et al., 1998). Some reports suggest potential roles for Brca2 in cell cycle regulation through its association with other proteins such as Brca2-associated factor 35 (Marmorstein et al., 2001), PIK1 (Lee et al., 2004), BCCIP-α (Liu et al., 2001), DSS1 (Marston et al., 1999), and Smad3 (Preobrazhenska et al., 2002). There have been several studies linking Brca2 with checkpoint regulation (Futamura et al., 2000; Lee et al., 1999; McKeon, 1999), although the results here can be conflicting as well. Brca2 is known to interact with mitotic checkpoint regulating proteins such as hBUBR1 (Futamura et al., 2000; Lee et al., 1999), although others have demonstrated that *Brca2*-deficient cells maintain the G1/S and G2/M cell cycle checkpoints (Morimatsu et al., 1998).

This wide variation in results and the number of conflicting reports illustrates how much uncertainty there is regarding Brca2's role in regulating cell growth. Much of this uncertainty could be related to cell type, tissue-specific, or developmental differences in function or perhaps differences in relation to the particular mutation being studied. To date, most studies looking for Brca2's role in cell growth have been conducted in vitro with cells not derived from mammary epithelium. The *Brca2*-mutant mice used in our laboratory lack just the terminal exon 27 of *Brca2* but still exhibit a high incidence of neoplasia, particularly carcinomas (McAllister et al., 2002). Here, we use these *Brca2*-mutant mice ($Brca2^{-/-}$) along with *p53*-mutant mice ($p53^{-/-}$) and double-mutant mice (dKO) carrying both mutations to

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