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Commentary

Are *Trp53* rescue of *Brca1* embryonic lethality and *Trp53*/*Brca1* breast cancer association related?

Kimberly A McAllister and Roger W Wiseman

Laboratory of Women's Health, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

Correspondence: Dr Kimberly A McAllister, PhD, Research Fellow, Laboratory of Women's Health, MD C4-06, Rall Building, National Institute of Environmental Health Sciences, NIH, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA. Tel: +1 919 541 3229; fax: +1 919 541 3720; e-mail: mcallis2@niehs.nih.gov

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Abstract

Brca1 is involved in multiple biological pathways including DNA damage repair, transcriptional regulation, and cell-cycle progression. A complex pattern of interactions of *Brca1* with *Trp53* has also emerged. Xu and coworkers found that haploid loss of *Trp53* significantly reduces the embryonic lethality observed in mice with a homozygous in-frame deletion of *Brca1* exon 11. They report that widespread apoptosis correlates with the embryonic lethality resulting from this homozygous Δ11 *Brca1* mutation. A mechanism responsible for *Brca1*-associated carcinogenesis is proposed. These experiments extend our knowledge of a complex *Brca1*/*Trp53* relationship. However, the precise mechanisms through which *Brca1* interacts with *Trp53* to suppress mammary tumor formation have yet to be elucidated.

Keywords: apoptosis, *Brca1*, breast cancer, *Trp53*, tumorigenesis

Introduction

Brca1 appears to have a role in multiple complex biological pathways including DNA damage repair, transcriptional regulation, and cell-cycle checkpoint control. *Brca1* is thought to function as a caretaker that is responsible for maintaining genomic integrity [1]. Loss of *Brca1* function has been shown to lead to defective DNA repair, to defective G_2-M cell-cycle checkpoints, to increased apoptosis, and to genomic instability. This generalized genetic instability is thought to lead to the accumulation of additional mutations that eventually allow *Brca1*-deficient cells to undergo neoplastic transformation (reviewed in [2]). Perhaps the most intriguing role of *Brca1*, however, is its complex interaction with *Trp53*.

The recent study by Xu *et al*. attempts to clarify our understanding of the complicated *Trp53*/*Brca1* relationship [3]. Substantial evidence suggests that *Brca1* and *Trp53* appear to be linked in their roles as tumor suppressor genes. *Brca1* and *Trp53* are coordinately regulated in their gene expression [4]. *Brca1* physically associates with *Trp53* and stimulates its transcriptional activity [5,6]. The *Trp53* protein appears to regulate *Brca1* expression levels [7], while *Brca1* in turn upregulates *p21* [8]. *Brca1* loss therefore triggers the *Trp53-Cdkn1a*-mediated cell-cycle checkpoint and corresponding cell death [9]. Finally, *Trp53* loss appears to enhance *Brca1*-linked tumorigenesis [10–12].

Trp53 rescue of *Brca1* embryonic lethality

The specific role of *Trp53* in rescue of embryonic lethality of mice with various *Brca1* mutations remains unclear. Earlier studies have shown that *Trp53* and *Cdkn1a* deficiencies can extend the development of several severe *Brca1* mutations [13–15]. Xu *et al.* [3] found that haploid loss of *Trp53* significantly rescues the embryonic lethality resulting from a $Brca1^{\Delta 11/\Delta 11}$ mutation. These $Brca1^{\Delta 11/\Delta 11}$ embryos normally die during late gestation in a wild-type

Trp53 background. With p53 haploinsufficiency, however, approximately 80% of the expected *Brca1*$^{\Delta 11/\Delta 11}$*Trp53*$^{+/-}$ mice survive based on Mendelian ratios for offspring from a double heterozygous cross. Unfortunately, Xu *et al*. [3] failed to note that the *Brca1* and *Trp53* loci reside within 20 cM of each other on mouse chromosome 11. These loci are therefore closely linked and do not recombine independently, as they would if located on different chromosomes. To determine the predicted genotypic distribution of offspring, the phase of the mutant *Brca1* and *Trp53* alleles on the parental chromosomes used in this intercross must be known. The expected numbers of specific genotypes in the offspring will differ widely depending on whether the mutant *Brca1* and *Trp53* alleles were present in cis versus trans configurations in the parental chromosomes.

Two other groups have described mice with related hypomorphic mutant *Brca1* alleles that remove all or part of exon 11 while preserving the Δ11 exon splice variant that produces a truncated *Brca1* protein. Cressman *et al.* [16] described three mice homozygous for both a mutant *Trp53* and a *Brca1* alteration that normally results in late embryonic lethality in the homozygous state. These authors propose that other genetic factors in addition to *Trp53* loss may be required for the survival of these *Brca1*-mutant mice. It is interesting to speculate that DBA/2-specific alleles may be involved since the DBA/2 genetic background also appears to enhance survival of hypomorphic *Brca2* mutants [17,18].

Ludwig *et al*. have generated mice with a related hypomorphic *Brca1* mutation [19], which was viable in particular genetic backgrounds in the absence of any additional *Trp53* mutation. This exon 11 mutation was predicted to lead to the truncation of the *Brca1* protein after the first 924 amino acids. This mutant *Brca1* protein is unique since it retains the nuclear localization signals in exon 11 as well as a proposed *Trp53* interaction domain between residues 224 and 500 [2,5]. However, a Δ11 splice variant protein is also still expressed in these mice as with mutants from the other groups. From original intercrosses between 129/Sv × C57BL/6J heterozygous parents, Ludwig *et al*. found only 4% homozygous *Brca1* mutants compared with the expected 25%. After backcrossing with 129/Sv mice or outcrossing to the MF1 strain, however, these investigators reported complete restoration of Mendelian ratios. These *Brca1* mutant mice thus appeared to have a more complete rescue of embryonic lethality based on genetic background differences alone than Xu *et al.* were able to obtain by introducing *Trp53* haploinsufficiency. Unfortunately, Xu *et al*. did not provide any information about the genetic background of their mutant mice. This dramatic difference in explanations for the embryonic lethality 'rescue' of these similar *Brca1* mutations is difficult to reconcile.

Xu *et al*. state that a major finding of the study was the ability to demonstrate the widespread apoptosis of *Brca1*-deficient embryos and the lack of this apoptosis in the *Brca1* mutants with *Trp53* mutations [3]. They propose that the apoptotic process normally triggered by *Brca1* loss does not occur with *Trp53* haploinsufficiency due to loss of the critical G_1-S checkpoint control. Cells with *Brca1* mutations that have accumulated DNA damage are therefore allowed to proliferate rather than undergo apoptosis. Xu *et al*. suggest this difference in apoptosis is a plausible explanation for the rescue of the embryonic lethality of these *Brca1* mutants. However, the rescue of *Brca1*$^{\Delta 11/\Delta 11}$ embryos did not occur when single downstream pathways of p53 were disrupted by eliminating either *Bax* or *Cdkn1a*.

Mammary tumorigenesis association with combined *Trp53*/*Brca1* deficiency

Multiple studies have now suggested that *Trp53* loss accelerates mammary tumor formation when associated with *Brca1* mutations. Although quantitative data was not presented in this report, Xu *et al*. [3] state that most of their female *Brca1*$^{\Delta 11/\Delta 11}$ mice that carried a *Trp53* mutation developed mammary tumors with loss of the remaining *Trp53* allele by 6–12 months of age. Ludwig *et al.* [13] found that many of their homozygous *Brca1*-mutant mice also developed mammary tumors as well as a wide variety of other tumors. They also investigated whether *Trp53* deficiency affected this *Brca1*-associated tumorigenesis, and observed accelerated tumorigenesis in mice carrying both mutations.

Trp53 hemizygosity allowed mice with the *Brca1* mutation developed by Cressman *et al*. [11] to develop a few mammary tumors after exposure to ionizing radiation, although these results did not achieve statistical significance. In contrast, mice with this *Brca1* mutation on a wild-type *Trp53* background did not develop mammary tumors. Finally, there was a decreased latency and increased incidence of mammary tumor formation in conditionally mutant *Brca1* mice that had a *Trp53* mutation [12,20]. A high frequency of *Trp53* mutations has also been observed in human BRCA-linked tumors. Some of these *Trp53* mutations are unique or unusual, suggesting that the tumorigenesis pathway mediated by combined *Brca1*/*Trp53* mutations may be different to the pathway for other *Trp53*-mediated tumorigenesis, such as what is observed in Li–Fraumini syndrome [21,22].

Biological pathway of the *Trp53*/*Brca1* interaction

Xu *et al.* attempted to further elucidate the molecular interaction between *Trp53* and *Brca1* that might lead to tumorigenesis in the *Brca1*$^{\Delta 11/\Delta 11}$ mice [3]. *Trp53* was found to have decreased stability in the *Brca1*$^{\Delta 11/\Delta 11}$ background after treatment with exogenous DNA damaging agents. Increased *Mdm2* levels and decreased phospho-

rylation of *Trp53* at Ser18 in γ-irradiated *Brca1*$^{\Delta 11/\Delta 11}$ mutant cells were suggested to be the primary causes for the decreased stability of *Trp53* in these cells. Xu *et al*. propose that this decreased stability of *Trp53* after stress-induced DNA damage might allow additional mutations to accumulate. Ultimately, such genetic alterations may allow the proliferation defect of *Brca1* deficiency to be overcome, and *Brca1*-associated tumorigenesis will result.

Conclusions

Although the report by Xu *et al.* has contributed to our understanding of the *Brca1*/*Trp53* relationship, a number of key questions remain to be addressed.

Why a rescue of *Brca1*-deficient embryonic lethality with *Trp53*?

The authors propose the substantial difference found in apoptosis between *Brca1*$^{\Delta 11/\Delta 11}$ mutant cells with or without *Trp53* mutations is a logical explanation for the rescue of the embryonic lethality of these *Brca1* mutants. However, several studies suggest that *Brca1* mutant embryos in which the *Trp53*-mediated apoptotic pathway is intact do not necessarily have an increase in apoptosis [14,16]. In addition, the rescue of *Brca1*$^{\Delta 11/\Delta 11}$ embryos did not occur when disrupting single downstream pathways of *Trp53*. This suggests that the process through which *Trp53* might allow for an extension of survival of *Brca1* mutants must involve either multiple *Trp53*-mediated pathways or additional genetic pathways altogether.

What is the relationship between BRCA1 and *Trp53* mutations that leads specifically to mammary tumor development?

The basis for the tissue specificity for *Brca1*/*Trp53* tumorigenesis remains unknown. It is interesting that the incidence of mammary tumors in *Trp53*-deficient mice appears to be dependent on genetic background strain [23]. This suggests that different sets of modifier loci in different inbred strains of mice impact the effect of the loss of *Trp53* in specific cell types or tissues. Other studies have shown that the type of tumor resulting from *Trp53* loss can differ dramatically depending on the tissue type and the oncogenic pathway involved [2]. In mammary tumorigenesis, it has been suggested that BRCA1 and *Trp53* deficiencies cooperate only with exposure of the mammary gland to DNA damage [11]. Alternatively, a lack of downstream mediators in tissues other than ovarian and mammary glands has been suggested to explain the tissue-specificity of *Brca1*-associated tumorigenesis [2].

Are the molecular mechanisms for the *Trp53* rescue of *Brca1* embryonic lethality and the *Trp53*-mediated acceleration of *Brca1*-induced mammary tumorigenesis the same?

Even if one assumes that *Trp53*-mediated apoptosis is significantly relevant for the mechanism of *Trp53* rescue of *Brca1* lethality, it is unclear how this *Trp53* mechanism relates to tumorigenesis due to *Trp53* loss. Although some studies clearly demonstrate the association of loss of *Trp53* with loss of apoptosis in developing tumors, other studies do not find this correlation, suggesting that p53 loss might alter tumor progression by different mechanisms in different cell types or tumor pathways [24,25]. In fact, some evidence suggests that the multiple functions of *Trp53* as a tumor suppressor, apoptosis inducer, and cell-cycle regulator may be entirely independent from each other [26]. Future directions to begin to establish the downstream partners and pathways involved in *Brca1*/*Trp53* tumorigenesis will clearly be necessary to answer these questions.

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