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

Abstract
Brca1 is involved in multiple biological pathways including DNA damage repair, transcriptional regulation, and cell-cycle checkpoint control. 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 G2-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 Δ11/Δ11 mutation. These Brca1 Δ11/Δ11 embryos normally die during late gestation in a wild-type...
Trp53 background. With p53 haploinsufficiency, however, approximately 80% of the expected Brca1Δ11/Δ11 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 G1-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 Brca1Δ11/Δ11 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 Brca1Δ11/Δ11 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 BRCA1-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 Brca1Δ11/Δ11 mice [3]. Trp53 was found to have decreased stability in the Brca1Δ11/Δ11 background after treatment with exogenous DNA damaging agents. Increased Mdm2 levels and decreased phospho-
rylation of Trp53 at Ser18 in γ-irradiated Brca1∆11/Δ11 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∆11/Δ11 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∆11/Δ11 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.

Acknowledgements
The authors thank Dr Cynthia Afshari and Dr Deborah Swope for the critical review of this manuscript.

References
1. Kinzler KW, Vogelstein B: Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 1997, 386:761-763.
2. Deng C-X, Brodie SG: Roles of BRCA1 and its interacting proteins. BioEssays 2002, 22:728-737.
3. Xu X, Qiao W, Linke SP, Cao L, Li W-M, Furth PA, Harris CC, Deng C-X: Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle, and tumorigenesis. Nat Genet 2001, 28:266-271.
4. Ouchi T, Monteiro ANA, August A, Aaronson SA, Hanafusa H: BRCA1 regulates p53-dependent gene expression. PNAS 1998, 95(5):2302-2306.
5. Zhang HB, Somasundaram K, Peng Y, Tian H, Zhang HK, Bi DK, Weber BL, el-Deiry WS: BRCA1 physically associates with p53 and stimulates its transcriptional activity. Oncogene 1998, 16(13):1713-1721.
6. Chai YL, Cui JQ, Shao NS, Reddy ESP, Rao VN: The second BRCT domain of BRCA1 protein interacts with p53 and stimulates transcription from the p21(WAF1/CIP1) promoter. Oncogene 1999, 18(1):263-268.
7. Andres JL, Saifin F, Turkel GJ, Wang J-A, Twu N-F, Yuan R-Q, Lamazus K, Goldberg ID, Rosen EM: Regulation of BRCA1 and BRCA2 expression in human breast cancer cells by DNA-damaging agents. Oncogene 1998, 16:2229-2241.
8. MacLachlan TK, Dash BC, Dicker DT, el-Deiry WS: Repression of BRCA1 through a feedback loop involving p53. J Biol Chem 2000, 275(41):31869-31875.
9. Somasundaram K, Zhang H, Zeng YX, Houyras Y, Peng Y, Zhang H, Wu GS, Licht JD, Weber BL, el-Deiry WS: Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor p21WAF1/CIP1. Nature 1997, 389:187-190.
10. Greenblatt MS, Chappuis PO, Bond JP, Hamel N, Foulkes WD: TP53 mutations in breast cancer associated with BRCA1 or BRCA2 germ-line mutations: Distinctive spectrum and structural distribution. Cancer Res 2001, 61(10):4092-4097.
11. Cressman VL, Backlund DC, Hicks EM, Gowen LC, Godfrey V, Koller BH: Mammary tumor formation in p53- and BRCA1-deficient mice. Cell Growth Differ 1999, 10:1-10.
12. Xu X, Wagner K-U, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng C-X: Conditional mutant of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. Nat Genet 1999, 22:37-42.
13. Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A: Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. Genes Dev 1997, 11(10):1226-1241.
14. Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmair A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J, Mak TW: The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. Cell 1996, 88:1009-1023.
15. Hakem R, de la Pompa JL, Eli A, Potter J, Mak TW: Partial rescue of Brca1(5-6) early embryonic lethality by p53 or p21 null mutation. Nat Genet 1997, 16(3):298-302.

16. Cressman VL, Backlund DC, Avrutskaya AV, Leadon SA, Godfrey V, Koller BH: Growth retardation, DNA repair defects, and lack of spermatogenesis in BRCA1-deficient mice. Mol Cell Biol 1999, 19:7061-7075.

17. Connor F, Bertwistle D, Ree PJ, Ross GM, Swift S, Grigorieva E, Tybulewicz VLJ, Ashworth A: Tumorigenesis and a DNA repair defect in mice with a truncating Brca2 mutation. Nat Genet 1997, 17:423-430.

18. Friedman LS, Thistlewaite FC, Pael KJ, Yu VPCC, Lee H, Venkitaraman AR, Abel KJ, Carlton MBL, Hunter SM, Colledge WH, Evans MJ, Ponder BAJ: Thymic lymphomas in mice with a truncating mutation in Brca2. Cancer Res 1998, 58:1338-1343.

19. Ludwig T, Fisher P, Ganesan S, Efstratiadis A: Tumorigenesis in mice carrying a truncating Brca1 mutation. Genes Dev 2001, 15:1188-1193.

20. Brodie SG, Xu X, Qiao W, Li WM, Cao L, Deng CX: Multiple genetic changes are associated with mammary tumorigenesis in Brca1 conditional knockout mice. Oncogene 2001, 20:7514-7523.

21. Crook T, Brooks LA, Crosland S, Osin P, Barker KT, Waller J, Philip E, Smith PD, Yulug I, Petro J, Parker G, Alliday MJ, Crompton MR, Gusterson BA: P53 mutation with frequent novel codons but not a mutator phenotype in BRCA1- and BRCA2-associated breast tumours. Oncogene 1998, 17:1681-1689.

22. Smith PD, Crosland S, Parker G, Osin P, Brooks L, Waller J, Philip E, Crompton MR, Gusterson BA, Alliday MJ, Crook T: Novel p53 mutations selected in BRCA-associated tumours which dissociate transformation suppression from other wild-type p53 functions. Oncogene 1999, 18:2451-2459.

23. Kuperwasser C, Hurlbut GD, Kittrell FS, Dickinson ES, Laucirica R, Medina D, Naber SP, Jerry DJ: Development of spontaneous mammary tumors in BALB/c p53 heterozygous mice. A model for Li–Fraumeni syndrome. Am J Pathol 2000, 157:2151-2159.

24. Symonds H, Kral L, Remington L, Saenz-Robles M, Lowe S, Jacks T, Van Dyke T: p53-dependent apoptosis suppresses tumor growth and progression in vivo. Cell 1994, 78:703-711.

25. Jones JM, Attardi L, Godley LA, Laucirica R, Medina D, Jacks T, Varmus HE, Donehower LA: Absence of p53 in a mouse mammary tumor model promotes tumor cell proliferation without affecting apoptosis. Cell Growth Differ 1997, 8:829-838.

26. Macleod KF, Jacks T: Insights into cancer from transgenic mouse models. J Pathol 1999, 187:43-60.