Double indemnity: p53, BRCA and cancer

p53 mutation partially rescues developmental arrest in *Brca1* and *Brca2* null mice, suggesting a role for familial breast cancer genes in DNA damage repair.

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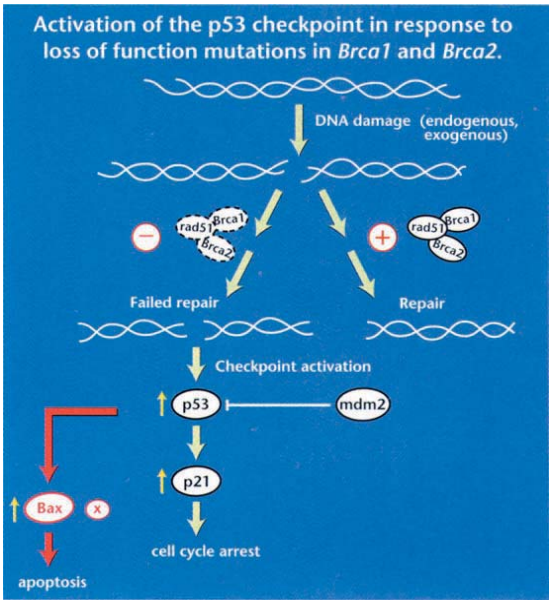
THE PAST SEVERAL years have seen an explosion of information in the field of cancer genetics, both with regard to the identity of genes that are mutated in sporadic forms of the disease as well as those that are inherited in mutant form, giving rise to a familial predisposition to cancer. The goal of this research is two-fold: (1) to decipher the series of changes in the regulation of cellular proliferation that underlie tumorigenesis and (2) to characterize the plethora of germline mutations (or polymorphisms) that increase an individual's lifetime risk of cancer. Of particular importance has been the recent identification of two genes whose function is critical to the prevention of breast cancer. Women who inherit a single defective copy of either of these tumor suppressor genes, termed *BRCA1* (ref. 1) and *BRCA2* (ref. 2), have a significantly increased lifetime breast cancer risk compared to the general population. In addition, *BRCA1* mutations predispose to ovarian cancer and *BRCA2* to prostate cancer. Interestingly, although inherited mutations in *BRCA1* and *BRCA2* are responsible for a high percentage of familial breast cancer cases, neither gene is clearly involved in the development of sporadic cancers.

In order to understand the tumor suppressive effects of the *BRCA1* and *BRCA2* genes, which are structurally unrelated, researchers have attempted to determine their functions through a combination of cell biological and biochemical methods. Although still somewhat controversial[3], the BRCA1 protein is believed to be localized primarily to the nucleus[4], and both BRCA1 and BRCA2 have been implicated in transcriptional regulation[5,6]. More consistent with a DNA repair function for BRCA1 and BRCA2, each has been shown to interact with the Rad51 protein[4,7]. A homologue of bacterial RecA and a gene required for a proper gamma irradiation response in yeast[8,9], Rad51 has been implicated in the regulation of recombination[4] and double-stranded DNA repair[10] in mammalian systems.

Another powerful method for establishing gene function, as well as for constructing animal models of human genetic diseases, is gene targeting in mice. Mouse strains engineered to be heterozygous for a mutation in a given tumor suppressor gene are the genetic analogues of humans affected by one of the familial cancer syndromes caused by inherited mutations in that gene. It is hoped that these animals exhibit the same sort of cancer predisposition as their human counterparts, in order to study the details of disease development as well as potential treatments or prevention strategies[11]. To date, however, mouse strains heterozygous for a mutation in either *Brca1* or *Brca2* do not exhibit clear tumor predisposition, either in the mammary gland or anywhere else[7, 12–15]. Thus, for these tumor suppressor genes, as has been seen for several others, there are clear species-specific differences in the consequences of inherited mutations.

In order to establish the functional requirements for tumor suppressor genes in embryogenesis, the *Brca1* and *Brca2* mutations have been crossed to homozygosity. In both cases, homozygous mutant embryos were found to fail very early in development: approximately day 5–6 of gestation (E5–6) for Brca1 (refs. 12–14) and about E7.5–8.5 for Brca2 (refs. 7,14,15). Thus, in addition to their importance in tumor development (at least in humans), these genes are also required for embryonic development to proceed to completion (at least in the mouse). Surprisingly, embryos lacking Brca1 or Brca2 function show reduced levels of cellular proliferation[12–15], which on the surface would seem incompatible with the tumor suppressive function of these genes. Furthermore, *Brca1* null embryos exhibit increased expression of the cell cycle inhibitor $p21^{waf1/cip1}$ (ref. 12), a gene upregulated by the p53 tumor suppressor gene[16] as part of the G1-phase growth arrest response following DNA damage[17,18]. Again, loss of function of Brca1 in this embryonic setting appears to activate a growth arrest pathway, rather than causing the unrestrained cell proliferation associated with cancer.



This model proposes that Rad51, Brca1 and Brca2 act as a complex to repair damaged DNA. Thus, mutations in any of these genes would lead to the accumulation of DNA damage and the subsequent activation of a checkpoint mechanism, resulting in p53 activation and the upregulation of the p53-responsive gene, *p21*. Increased p21 levels inhibit cyclin-dependent kinases, the enzymes that catalyze progression through the cell cycle, resulting in cell cycle arrest. Genes other than p21, such as Bax, are also regulated in response to p53 activation in other circumstances, leading to apoptosis. In addition to Bax, undiscovered genes (X), are thought to be implicated in p53-mediated apoptosis. The activity of p53 is negatively- regulated by mdm2 and loss of mdm2 results in embryonic lethality in the mouse that is completely rescued by a p53 mutation[23,24].

This apparent paradox has been addressed recently by additional experiments in the mouse, and these lend strong support for a role for Brca1 and Brca2 in the response to DNA damage. Specifically, two groups, reporting in a recent issue of *Genes and Development*[14] and in the current issue of *Nature Genetics*[19], demonstrate a partial rescue of the Brca1 and Brca2 developmental phenotypes through simultaneous mutation of p53. Double mutant embryos survive an

additional one to two days of gestation — *Brca1* $^{-/-}$, *p53*$^{-/-}$ until ~E8.5–9.5 (refs, 14,19) and *Brca2*$^{-/-}$, *p53*$^{-/-}$ until E9–10 (ref.14). Therefore, the early lethality caused by mutation of *Brca1* or *Brca2* is dependent on p53 function. Moreover, Hakem and colleagues[19] further show that mutation of *p21* can also ameliorate the *Brca1* null phenotype; again, double mutant embryos survive until ~E8.5–9.5. One interpretation of these findings is that the absence of Brca1 or Brca2 function in the early embryo results in a failure to repair DNA damage, which presumably arises as a consequence of the rapid rate of replication at this stage of development (see figure). The unrepaired DNA damage then activates the p53-dependent growth arrest response and a cessation of embryonic development ensues. Simultaneous elimination of p53 function (or that of it's growth-arrest effector, p21) abrogates the arrest response and allows continued cellular proliferation. The subsequent death of the double mutant embryos may result from the accumulation of a degree of genetic damage that is incompatible with further development.

These findings show striking parallels with previous work on *rad51* knockout mice[10, 20]. Embryos deficient in *rad51* fail in gestation at the same stage as *Brca1* null embryos and also exhibit reduced cellular proliferation[10]. Furthermore, embryos lacking both *Rad51* and *p53* survive longer in gestation, until ~E8.5–9.5 (ref. 10). Once again, the failure to properly repair DNA damage in the early embryo in the absence of Rad51 appears to activate a p53-dependent growth arrest response, which can be partially overcome with *p53* mutation. Given the reported physical interaction between Rad51 and both BRCA1 (ref. 4) and Brca2 (ref. 7) along with the similarities in the knockout phenotypes, it seems quite likely that these three proteins act in concert during the normal response to DNA damage (see figure). Finally, both *Brca2*$^{-/-}$ (ref. 7) and *rad51*$^{-/-}$ (ref. 10) embryos exhibit increased sensitivity to radiation, directly implicating these proteins in DNA damage repair.

How might these murine developmental phenotypes relate to mutation of *BRCA1* and *BRCA2* in human breast cancer development? On the one hand, as components of the DNA damage response pathway, these two genes would be required for the stability of genetic information, and their loss during tumorigenesis would be expected to result in an elevated mutation rate. This in turn would increase the likelihood of cells acquiring oncogenic mutations, ultimately leading to cancer. According to this model, the tumor-promoting effects of *BRCA1/BRCA2* mutations would be indirect, acting to lower the threshold to subsequent changes that would directly affect the growth properties of the cell. This scenario is reminiscent of other familial cancer syndromes, including hereditary nonpolyposis colon cancer and xeroderma pigmentosum, for which the responsible genes are critical components of the mismatch and nucleotide excision repair pathways, respectively. However, at least in the context of the early mouse embryo, mutation of *Brca1* or *Brca2* causes cell cycle arrest through activation of the p53-dependent checkpoint mechanism[14,19]. Thus, for *BRCA1/BRCA2* mutations to contribute to increased genomic instability in cancer, this checkpoint must either be rendered nonfunctional by prior mutation or be inactive in the relevant cell types of the adult. Intriguingly, a high percentage of familial breast cancers develop mutations in the *p53* gene[21].

Although the recent findings strongly support the involvement of BRCA1 and BRCA2 in the repair of DNA damage, many questions remain. For example, given such a universally important function, why do germline *BRCA1* and *BRCA2* mutations specifically predispose to breast and ovarian cancer? (Similar confusion surrounds colon cancer predisposition and mismatch repair deficiency). Also, what accounts for the absence of *BRCA1/BRCA2* mutations in sporadic forms of the disease? If the loss of these genes acts to lower the threshold to mutation, will there be any therapeutic value in reconstituting their function in tumors? Will mutations in other components of this DNA damage pathway contribute to familial breast cancer? And to what extent do the mutant phenotypes in mice relate to humans? As mentioned above, mice heterozygous for *Brca1* or *Brca2* mutations have not yet exhibited cancer predisposition[7,10–15]. Also, Boyd and colleagues[22] have described a woman from a breast cancer family who is homozygous for her *BRCA1* mutation. If this mutation is a null, then full loss of BRCA1 function must have quite different consequences in humans and mice, which could indicate somewhat different activities of the protein in the two species. Clearly, much additional research is needed to address these questions, but the recognition of a role for the familial breast cancer genes in DNA damage repair represents an important advance.

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