The BRCA1 suppressor hypothesis: An explanation for the tissue-specific tumor development in BRCA1 patients

The tumor suppressor gene *BRCA1* is required for the cellular response to DNA damage and homologous recombination, although the exact function it plays in these processes is unclear (Moynahan et al., 1999; Scully and Livingston, 2000). Consistent with the idea that BRCA1 plays a central housekeeping role within cells, mice lacking BRCA1 die very early in embryogenesis (E7.5 or earlier) due to extreme genomic instability and activation of the p53/p21 pathway (Gowen et al., 1996; Hakem et al., 1996; Ludwig et al., 1997). Despite its apparent central role in double-strand break repair, loss of *BRCA1* function appears to affect only a very specific and small subset of tissues in humans. Although a slight increase in predisposition of other cancer types may exist in patients carrying a germline mutation in the *BRCA1* gene, a dramatic increase in predisposition is observed only for cancer of the breast and ovary in these individuals (reviewed in Welcsh et al., 1998; Scully and Livingston, 2000; Rosen et al., 2001). Most mutations in *BRCA1* thus far represent single base pair deletions, insertions or large genomic rearrangements likely causing a complete loss of function of *BRCA1* due to the resulting frameshifts (Neuhausen and Ostrander, 1997; Blackwood and Weber, 1998), excluding the possibility that these *BRCA1* mutations are weak hypomorphic alleles. The phenotypes associated with these truncations are in fact expected to be at least as deleterious as the *BRCA1* mutations made thus far in mice.

Why BRCA1 loss is not observed in sporadic breast and ovarian cancer

The role of BRCA1 in breast cancer appears to be limited to familial cases. The absence of *BRCA1* mutations in sporadic breast and ovarian tumors has been treated as a surprising aspect of BRCA1 biology. However, given the properties of familial BRCA1 disease, homozygous mutations in *BRCA1* are actually expected to be relatively rare in sporadic tumors. The reasoning for this is as follows: women who inherit a mutant allele of *BRCA1* typically develop unilateral breast cancer with a mean age of incidence of 50–70 years, depending on the study. The overall penetrance of cancer in individuals inheriting one mutant *BRCA1* allele is 85% for breast cancer and 65% for ovarian cancer during their lives (Easton et al., 1995). For a sporadic tumor to evolve from a $BRCA1^{+/+}$ individual, the tumor would first have to lose one *BRCA1* allele spontaneously to become $BRCA1^{+/-}$, at which point those cells would have approximately the same probability of becoming a tumor as equivalent cells from *BRCA1* heterozygous patients. Thus, spontaneous tumors due to BRCA1 require an additional hit relative to those derived from *BRCA1* heterozygotes and are correspondingly less frequent.

The frequency of tumor occurrence of the $BRCA1^{-/-}$ genotype arising spontaneously from $BRCA1^{+/+}$ individuals is, at least to some extent, the frequency of conversion from $BRCA1^{+/+}$ to $BRCA1^{+/-}$ for an individual cell, i.e., the additional hit, multiplied by the frequency of tumorigenisis in $BRCA1^{+/-}$ patients (0.85 for breast tumors). An exact prediction of the expected percentage of sporadic cases of $BRCA1^{-/-}$ tumors of the breast and ovary is difficult to calculate for several reasons. The spontaneous rate of forward mutagenesis, generally thought to be between 1 in 10^{-6} to 1 in 10^{-7} mutations/generation for normal cells, is affected by the presence or absence of prior genetic instability mutations. Furthermore, loss of heterozygozity of *BRCA1* may only cause tumor formation efficiently when it occurs during a certain developmental window. The timing of occurrence of LOH is necessarily delayed for spontaneous versus familial disease by the requirement for a prior mutation to occur ($BRCA1^{+/+}$ to $BRCA1^{+/-}$). The frequency with which a spontaneous $BRCA1^{-/-}$ tumor is observed among breast cancer samples also depends on the number and frequency of mutations other than *BRCA1* that cause breast cancer. The more different types of genetic alterations cause breast cancer and the more frequently such mutations occur, the less frequent a tumor due to any one particular cause will be detected. The fact that 12% of women develop breast cancer in their lifetime, a high number, means that a relatively large number of tumors would have to be screened to identify the rare tumors due to spontaneous BRCA1 loss.

Given the penetrance and relatively late onset of tumorigenesis in *BRCA1* heterozygotes, spontaneous $BRCA1^{-/-}$ tumors are expected to be relatively rare. This line of reasoning provides a general prediction for the relationship between inherited and sporadic tumor suppressors: if the cancers caused by familial tumor suppressors occur early in the life of an individual who inherits a mutant allele and with high penetrance, mutations should also be observed in sporadic cancers; if the familial-inherited cancers arise later in life and with relatively low penetrance, the sporadic forms are predicted to occur only rarely because they require an additional hit.

Tissue specificity of tumorigenesis in *BRCA1* heterozygous individuals

Why mutations in a generic DNA repair gene should show such striking tissue specificity has been a source of much speculation (reviewed in Welcsh et al., 1998; Scully and Livingston, 2000; Rosen et al., 2001). The concept of "redundancy" has been put forth as one explanation of this phenomenon. It has been proposed that most tissues have alternative ways of performing BRCA1's tumor suppressing functions. Therefore, loss of BRCA1 in tissues with backup mechanisms has less of an effect than loss in the tissues such as breast and ovary without backup mechanisms. The embryonic defects observed in knockout mice described below argue against a general redundancy. Another plausible explanation involves the high proliferative activity in ovary and breast tissues. Cell proliferation in the absence of BRCA1 may lead to a higher mutation rate, thereby increasing the risk of acquiring a cancer-causing mutation. However, other tissues such as the colon also have high proliferation rates but do not show this high incidence of tumor formation in affected individuals. BRCA1 has been implicated in the regulation of transcription, and it is possible that it may regulate certain genes expressed only in the breast and ovaries. The altered expression of these transcripts would lead to an increase in neoplastic transformation through an as yet undefined mechanism (Welcsh et al., 1998). Alternatively, ovary- and breast-specific BRCA1 cofactors could cause transformation in the absence of BRCA1. Finally, at least one report has suggested that BRCA1 plays an inhibitory role in estrogen receptor (ER) signaling that could help explain the tissue specificity (Fan et al., 2001). A fact relevant to this explanation is that most $BRCA1^{-/-}$ tumors lack ER expression. It is currently unclear if

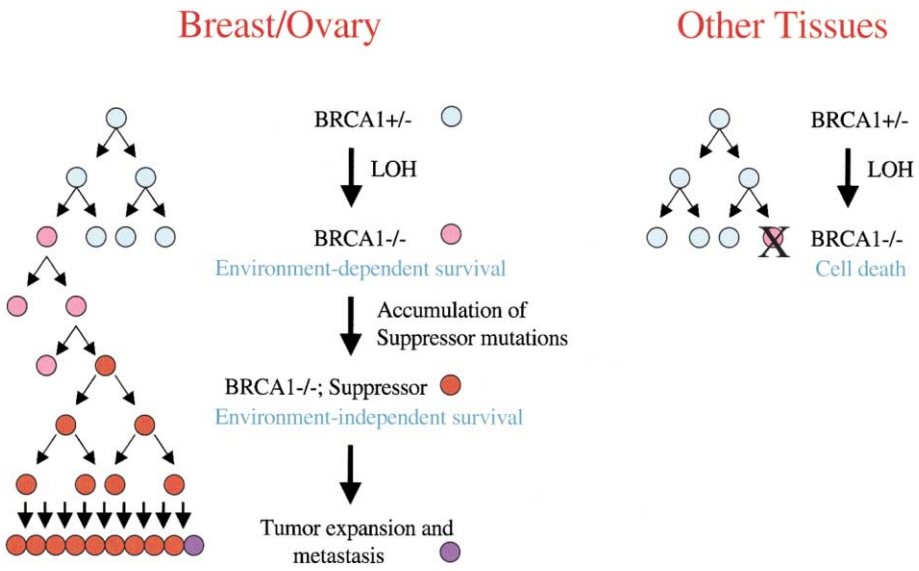

Figure 1. A schematic representation of the BRCA1 suppressor hypothesis as an explanation for breast and ovarian tissue specificity of cancer in *BRCA1* heterozygous individuals

See text for explanation. LOH: loss of heterozygosity. Colored circles represent cells of particular genotypes. Changes in circle color indicate a change in genotype.

BRCA1$^{-/-}$ tumors arose from cells that never expressed ER or that lost ER expression during tumorigenesis. If these tumors never expressed ER, however, it would call this potential explanation into question.

The BRCA1 suppressor hypothesis

We would like to propose a new model for the tissue specificity of tumor formation in BRCA1 patients that relies on the essential nature of BRCA1 (Figure 1). We propose that because *BRCA1* is an essential gene, the loss of BRCA1 leads to cell death or a severe decrease in proliferation in tissues other than the breast and ovary, thus reducing the likelihood that additional mutations will occur that allow tumor formation. Only in the breast and ovaries are *BRCA1*$^{-/-}$ cells able to survive long enough for secondary mutations to occur that support cell proliferation. Thus, the reason why loss of BRCA1 causes tissue-specific tumor formation is not because most tissues have "redundant" ways of repairing DNA and/or promoting cell survival, but that only breast and ovary tissues are able to survive for a prolonged period of time in the absence of BRCA1.

This hypothesized increased survival may be due to genetic factors unique to these particular tissues. Such genetic factors might include the presence of additional DNA repair capacities in these cells or expression of BRCA1-related proteins capable of compensating for loss of certain BRCA1 functions. Other genetic factors could result in a reduced propensity to undergo DNA damage-induced cell death due to expression of higher levels of antiapoptotic genes.

It is also possible that it is the physiological environment in these tissues that promotes survival in the absence of BRCA1. For example, the presence of survival factors in the form of hormones may have a protective effect on individual cells, allowing *BRCA1*$^{-/-}$ cells to survive and proliferate for a prolonged period of time. Both the breast and ovaries are targets of estrogen and other hormones that have been shown to endow anti-apoptotic survival functions upon cells, sometimes in a cell nonautonomous manner (Gompel et al., 2000; Kousteni et al., 2001).

If the lethality caused by the loss of both *BRCA1* alleles is suppressed even temporarily, further proliferation and survival-promoting mutations would accumulate due to the extreme genomic instability caused by loss of BRCA1 function (Figure 1). Once cells have acquired additional survival-promoting genetic changes (suppressor mutations), they will rapidly evolve further to form a tumor. The acquisition of suppressor mutations that allow these cells to survive independent of the protective environment of the breast may be a key step in their ability to ultimately undergo metastasis.

The types of suppressor mutations that will allow clone expansion are unknown, but clues can be gleaned from experiments performed in mice. Mice homozygous for the *BRCA1* mutations die early in embryogenesis (Gowen et al., 1996; Hakem et al., 1996; Ludwig et al., 1997). However, when p21 or p53 are deleted in these mutant backgrounds, embryos survive several days longer and even to birth depending on the severity of the *BRCA1* mutation examined (Ludwig et al., 1997; Hakem et al., 1996; Cressman et al., 1999; Xu et al., 2001). These experiments and others indicate that the DNA damage response pathway is activated in the absence of BRCA1. Therefore, mutations in the DNA damage response pathway might be expected to suppress BRCA1 loss-of-function-associated cell lethality in non-breast and -ovary tissues and contribute to tumorigenesis.

Predictions from this hypothesis

There are a few key predictions resulting from this hypothesis. (1) Weaker alleles of *BRCA1* may be expected to show cancer predisposition in other tissues. Hypomorphic nonlethal alleles of *BRCA1* could survive in a homozygous state in tissues that do not have the protective environment of the breast and ovaries. The reduced genomic instability of these partial loss-of-function alleles might be offset by the greater number of cells in the body in which homozygous mutations can survive. A recent report lends support for this hypothesis. Mice homozygous for a hypomorphic truncated *BRCA1* allele (*BRCA1*^{tr}) are viable and show a strikingly broad tumor spectrum (Ludwig et al., 2001) including lymphomas, sarcomas, and adenomas/carcinomas of the breast, lung, and liver. Since we do not know the tumor spectrum of more deleterious *BRCA1* alleles in mice, we cannot determine if this hypomorphic allele extends the tumor spectrum, but it is consistent with the prediction put forth by our hypothesis. (2) Promoting survival of *BRCA1*$^{-/-}$ cells in tissues other than the breast and ovary by inhibiting apoptosis might lead to tumor formation in these tissues. As noted above, loss of p53, which leads to the inactivation of some apoptotic pathways, allows BRCA1 null animals to survive longer. Mice homozygous for the loss of exon 11, a hypomorphic allele of *BRCA1*, die between days E12 and E18 of gestation. Loss of only one allele of p53 suppresses this lethality (Xu et al., 2001),

but these mice also show an increase in tumor susceptibility in a wider range of tissues (Xu et al., 2001). Similarly, loss of components of the DNA damage checkpoint might suppress the lethality of *BRCA1*$^{-/-}$ cells and expand the tumor spectrum in *BRCA1* mutant mice.

BRCA1 is an unusual tumor suppressor in that it is virtually essential for cell viability due to its role in maintaining genomic integrity. Other genes with these general properties are likely to exist and give rise to a similar tissue distribution of tumorigenesis. In addition to *BRCA2* whose mutation results in early embryonic lethality in mice (Ludwig et al., 1997) and tissue-specific tumor occurrence in humans, there are other genes that could be members of this class of tumor suppressors. *RAD51*, which associates with *BRCA1* and *BRCA2*, is one such candidate. *RAD51*, like *BRCA1* and *BRCA2*, is required for embryonic development. However, thus far, *RAD51* has not been reported to be mutated in familial breast cancer. Perhaps other components of the homologous recombination repair pathway and proteins known to associate with BRCA1, such as those in the BASC complex (reviewed in Welcsh et al., 1998; Scully and Livingston, 2000; Rosen et al., 2001), may also be members of the *BRCA1* class of tumor suppressors, assuming that it is this aspect of BRCA1 function that is relevant for tumor prevention. Other essential genes involved in genomic stability like *ATR* and *CHK1* may be members of the *BRCA1* class of tumor suppressors. However, unlike in the case of *BRCA1* and *BRCA2* mutants, the early embryonic lethality of these mutants is not rescued by loss of p53 (Liu et al., 2000; Brown and Baltimore, 2000). This suggests that loss of ATR and CHK1 causes a more severe defect in DNA metabolism and/or checkpoint function than does loss of BRCA1. This more severe defect may not be rescued by a survival-promoting genetic or physiological environment. It is, however, tempting to speculate that hypomorphic alleles of these genes would show breast and ovary specificity for tumorigenesis.

In general, tissue-specific tumor suppressors gain their specificity by exploiting unique properties of their target tissues. Rb may exploit the dependency of retinal tissues for differentiation on the Rb pathway. Mutations in ATM, which is specifically required for the response to double-strand breaks, shows a high preponderance of leukemias and lymphomas, which are derived from cell types that undergo double-strand breaks in the course of developing their immune repertoire. We propose that essential genes like *BRCA1* and *BRCA2* adhere to this general paradigm and are likely to be exploiting a cell survival aspect of the breast and ovaries to gain their tissue specificity.

Implications of the suppressor hypothesis for therapy

If this suppressor hypothesis is correct, identifying the genetic and environmental factors that enhance survival of cells homozygous for *BRCA1* mutations will be a key aspect of the successful prevention and treatment of this cancer. For example, if estrogen is causing the supportive environment that allows *BRCA1* mutations to survive in the breast and ovary, then antiestrogen therapies should decrease the incidence of breast and ovarian cancers in patients carrying *BRCA1* mutations. This protective effect would be highest before a tumor has occurred. Estrogen has been shown to induce expression of other survival factors such as Igf1 and Wnt (Rutanen, 1998; Sassoon, 1999). It is possible that interfering with these proteins may provide a means of prevention that has fewer deleterious side effects than antiestrogen therapies. It should be noted that, although most *BRCA1*$^{-/-}$ tumors lack ER, the fact that estrogen can signal through these paracrine mechanisms would still allow estrogen to affect cells lacking the ER. A critical point with respect to possible therapies is that we hypothesize that the loss of the second *BRCA1* allele should be an early event in the initiation of breast cancer, well before the earliest onset of cancer in these patients. Therefore, any preventative therapies aimed at preventing the survival of *BRCA1*$^{-/-}$ cells would have to begin relatively early in life after the proposed protective environment in the breast is established.

If cell models can be identified that allow BRCA1 null cells to survive by the same mechanism as they do in the breast, these lines can be used to search for drugs that specifically inhibit their survival but do not affect *BRCA1*$^{+/+}$ cells. These drugs could open new avenues for preventative therapies and may also have palliative effects on early tumors in situ which may still benefit from the putative protective environment of the breast and ovaries.

The authors would like to note that the ideas put forth in this essay are purely speculative and are made for the sole purpose of spurring on research into this important area of biology. This essay was not meant to be a comprehensive review of the literature concerning BRCA1, and we apologize to our colleagues whose work we were not able to cover due to space constraints.

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