Breast cancer gene variants: separating the harmful from the harmless

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Individuals carrying a mutation in the breast cancer 1, early onset gene (*BRCA1*) are at increased risk of breast or ovarian cancer and thus are candidates for risk reduction strategies such as oophorectomy and mastectomy. A recurring problem in the clinic is that many detectable changes within the *BRCA1* gene produce subtle alterations to the protein that are not easily recognized as either harmful (loss-of-function) alleles or harmless and thus inconsequential polymorphisms. In this issue of the *JCI*, Chang, Sharan, and colleagues describe a novel system to evaluate human *BRCA1* alleles for in vivo function using BACs containing human *BRCA1* vectors in mouse cells and embryos (see the related article beginning on page 3160). This strategy should provide new avenues for clinicians to interpret results of genetic testing of *BRCA1* variants and for researchers to study the basic molecular mechanisms of BRCA1 function in in vivo model systems.

The problem of *BRCA* variants of unknown significance in genetic testing

Genetic testing for deleterious mutations in breast cancer 1, early onset gene (*BRCA1*) and *BRCA2* can provide key information to guide clinical decision making. Women who are heterozygous carriers of mutations in either gene have a 60%–80% lifetime risk of breast cancer and a 10%–40% lifetime risk of ovarian cancer (1), reflecting a very high penetrance. In the clinic, genetic testing for *BRCA1* and *BRCA2* mutations is offered to women in high-risk families and yields one of several possible results. The first is that a deleterious mutation is detected and those with such a mutation are counseled on risk reduction strategies such as breast MRI for early detection, chemoprevention, and prophylactic oophorectomy and mastectomy (2–4). In addition, therapies designed to exploit the DNA repair deficits in *BRCA*-mutated cells are now entering the clinic; early studies have shown that inhibition of poly(ADP-ribose) polymerase (PARP) is a potential therapeutic strategy for treating cancers arising in individuals with *BRCA1* or *BRCA2* mutations (5). Thus, since their respective initial discoveries in 1994 and 1995, basic investigations into BRCA1 and BRCA2 functions at the genetic, biochemical, and in vivo levels have begun to fulfill the promise of molecular cancer research by providing a means to accurately predict cancer risk and to provide tailored therapies either to prevent the development of malignancy or to treat it.

Another possible result of genetic testing is the identification of a variant of unknown significance (VUS). VUSs are sequence variations in a gene for which the effect of the sequence change on the function of the protein is not known; the change may result in loss of function and thus increased risk of cancer but also may be a benign polymorphism with no excess cancer risk. Most VUSs are single nucleotide substitutions (also called missense alleles) that result in a single amino acid change. Some missense mutations clearly alter the function of BRCA1, such as those that occur in the RING finger or BRCA1 C terminus (BRCT) domains or induce frameshifts by altering splice sites. Unfortunately, for most VUSs the effect on protein function is not known. Approximately 10% of individuals undergoing genetic testing for *BRCA1* and *BRCA2* mutations will be found to have a VUS (6), with higher rates of VUSs in populations of non-European descent, in which fewer individuals have been tested. Thus, women that already have considerable anxiety regarding their risk of malignancy are presented with ambiguous information when informed they harbor a *BRCA* VUS. Assignment of risk to VUS alleles consequently becomes a difficult and all too common scenario in the clinical setting.

Clinical approaches to the assessment of VUSs have been described (7), including testing in a family to determine whether there is cosegregation of the VUS with disease, as well as examining differences in prevalence of a VUS between cases and controls. Such approaches have limitations, and ultimately what matters most is whether a VUS results in a change in protein function.

To tackle this problem, researchers have employed a myriad of approaches. Most of these assays are indirect and, while informative, do not measure physiologic BRCA1 activity within the context of a mammalian cell. Data overwhelmingly link the tumor suppression activities of BRCA1 and BRCA2 to DNA repair by homologous recombination (HR) (8). This basic premise was revealed in landmark experiments by Scully and Livingston, and Sharan and Bradley demonstrating that BRCA1 and BRCA2 colocalize and biochemically interact with the RAD51 recombinase at DNA damage sites (9, 10). BRCA1 is, at least in part, a scaffolding protein that maintains multiple protein-protein interactions with other DNA repair proteins to positively influence HR, many of which are the product of cancer susceptibility genes themselves (11, 12). *BRCA1* alleles that disrupt these interactions are invariably impaired with respect to DNA damage response function and considered to be clinically significant. VUS alleles at the aminoterminal RING domain can also be readily ascertained in in vitro E3 ubiquitin ligase assays as a second means of determining whether a VUS results in functional impairment (Figure 1A). Confirmed cancer-causing *BRCA1* missense changes in the RING domain disrupt in vitro BRCA1 E3 ubiquitin ligase activity (13, 14). Interaction deficiency is, however, a limited means of analyzing *BRCA1* VUSs, given that many of these missense changes

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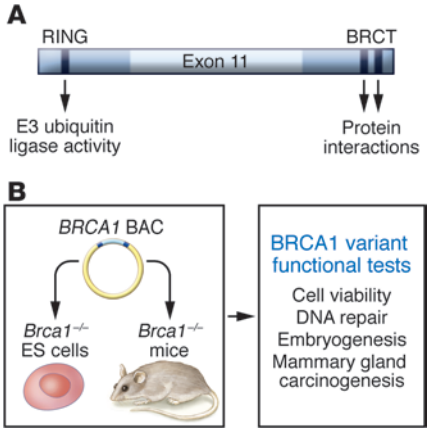

Figure 1

Methods for assessing the function of human *BRCA1* VUS alleles. (**A**) BRCA1 domain structure, depicting the amino terminal RING domain, exon 11–encoded region, and C-terminal BRCT repeats. Cancer-causing mutations and VUS changes occur in each of these regions. The influence of VUSs that occur in the RING and BRCT domains can be examined for effect on E3 ubiquitin ligase activity and protein interactions, respectively. (**B**) The human *BRCA1* BAC reconstitution system described by Chang, Sharan, and colleagues in this issue of the *JCI*, in which human *BRCA1* BAC DNA with any mutation can be introduced into mouse ES cells containing a conditional allele of *Brca1*, enabled in vitro investigation of VUSs anywhere within the human *BRCA1* gene and their effects on cell viability, DNA repair, embryogenesis, and mammary gland carcinogenesis (15). The authors validated their in vitro system with follow-up studies of known neutral and deleterious BRCA1 variants in mice.

map neither to the RING domain nor to protein interaction surfaces on BRCA1. Development of a facile system to ectopically express human BRCA1 under the control of its endogenous regulatory elements would be of obvious benefit to basic and translational studies involving VUS or synthetically designed *BRCA1* alleles.

Humanizing the mouse: a new approach to understanding *BRCA* VUSs

In this issue of the *JCI*, Chang, Sharan, and coworkers report an elegant approach to evaluating clinical and experimentally designed *BRCA1* missense alleles (15). BACs containing the human *BRCA1* gene and its requisite regulatory elements were engineered to contain a point mutation of interest and then introduced into mouse ES cells harboring a conditional allele of the mouse *Brca1* gene. This clever set of engineering steps enabled the investigators to delete the endogenous mouse *Brca1* allele and subsequently investigate human *BRCA1* VUS alleles for in vivo function (Figure 1B). Transgenic expression of this human *BRCA1* BAC in mice nullizygous for the mouse *Brca1* allele supported viability without a detectable phenotype through adulthood (16). Conversely, BRCA1-null mice die early in embryonic development. Ostensibly, human *BRCA1* BAC constructs contain the appropriate regulatory elements to express BRCA1 in the correct temporal and spatial manner, and the human BRCA1 protein fulfills all of the necessary functions in the mouse to successfully navigate the stringent criteria of embryonic and postembryonic development. Since cancer-causing mutations disrupt BRCA1 function, it is presumed that clinically significant VUSs would not support viability in this context.

The authors use this human *BRCA1* BAC reconstitution system to investigate 13 different *BRCA1* alleles in mouse ES cells, and 3 of these variants were selected for in vivo studies during mouse embryogenesis (15). Mouse Brca1 is required for viability of cultured ES cells as well as embryonic development, making rescue of lethality a convenient marker of human BRCA1 function. Initial testing of 3 known cancer-causing missense mutations at either the RING or BRCT domains revealed cell lethality upon *Cre* recombinase–mediated excision of the mouse *Brca1* gene, while a suspected neutral *BRCA1* variant, M1652I, restored viability at levels similar to those of wild-type human *BRCA1*. Similarly, M1652I was the only variant to rescue embryonic development. This initial validation was followed by testing of additional VUS alleles and phosphorylation-deficient *BRCA1* mutants for DNA damage response functions.

Several important concepts begin to emerge from this study (15). Clinically recognized deleterious missense mutations within the *BRCA1* RING and BRCT domains are associated with high cancer penetrance and in this model were inconsistent with cell viability, suggesting that BRCT interaction with its direct binding partners (Abraxas, Brip1/FANCJ, and CtIP) is essential for both viability and tumor suppression. It is somewhat surprising that an intermediate phenotype did not arise in this setting, given that *BRCA1* BRCT truncation–mutant mice survive 3–5 days longer during embryogenesis than do mice with a complete *Brca1* deletion (17). Moreover, mouse knockout experiments indicate that BRCA1 mutations within the exon 11–encoded region are capable of supporting viability and still confer tumor susceptibility (18). The striking correlation between the support of ES cell viability and tumor suppression in the human BAC reconstitution system may reflect an increased dependence of ES cells on HR-mediated DNA repair compared with other cell types. It will be interesting to determine whether support of ES viability is inextricable from tumor suppression for all *BRCA1* alleles in this BAC reconstitution system.

The authors also use their reconstitution system to shed light on basic questions regarding DNA damage–induced phosphorylation (15). The kinases ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) extensively phosphorylate BRCA1 after DNA damage (19, 20). How these phosphorylation events are initiated and their significance for BRCA1-dependent DNA damage responses are unknown. The authors reveal an unanticipated function of cyclin-dependent kinase 2–mediated (Cdk2-mediated) phosphorylation of BRCA1 at serine 1,497 as a potential gatekeeper for subsequent phosphorylations by ATM/ATR. Expression of the human *BRCA1* S1497A mutant strongly diminished subsequent ionizing radiation–induced (IR-induced) ATM/ATR-dependent phosphorylations on BRCA1 and conferred IR sensitivity. Surprisingly, HR-mediated DNA double-strand break repair remained intact, implying that ATM/ATR signaling through BRCA1 regulates the DNA damage response by other mechanisms. The molecular basis for this phenotype can in principle be addressed by functional experiments in the reconstituted ES cells and by affinity purification experiments of phospho-deficient BRCA1 proteins.

There are more than 800 *BRCA1* VUS alleles in the Breast Cancer Information Core database (http://research.nhgri.nih.gov/bic/), reflecting the enormity of genetic variation within this gene and the need to understand it for clinical benefit. The work of Chang, Sharan, and coworkers provides a new set of tools to tackle this very significant challenge (15) (Figure 1). In addition, BAC reconstitution approaches have the potential for application in the study of VUSs of other inherited cancer susceptibility genes, including *BRCA2* (21), *p53*, and the colorectal cancer–associated genes *MLH1* and *MSH2*. In each of these

situations, the power of molecular biology can be harnessed to separate the harmful variants from the harmless, allowing both patients and physicians to make appropriate clinical decisions.

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