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 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 BRCA1-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 mammary gland. 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 vitro in 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

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: J Clin Invest. 119:2895–2897 (2009). doi:10.1172/JCI40577.

Breast cancer gene variants: separating the harmful from the harmless

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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 died 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 unexpected function of cyclin-dependent kinase 2–mediated (Cdks2-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.

Acknowledgments
R.A. Greenberg gratefully acknowledges K08 award 1K08CA106597-01 from the National Cancer Institute, an American Cancer Society Research Scholar Grant, the Sidney Kimmel Foundation Scholar Award, The Mary Kay Ash Foundation Translational Innovation Award, and funds from the Abramson Family Cancer Research Institute. S.M. Domchek was supported by the Cancer Genetics Network (HHSN21620074400C), the Marjorie Cohen Foundation, and the QVC Network — Fashion Footwear Association of New York.

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