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BRCA1, Everything But the RING?

Roger A. Greenberg

Department of Cancer Biology and Department of Pathology, Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, 421 Curie Boulevard, Philadelphia, PA 19104–6160, USA. rogergr@mail.med.upenn.edu

Abstract

Genetically engineered mouse models reveal essential functions of the BRCA1 protein in tumor suppression.

Since its discovery in 1994, familial breast and ovarian cancer susceptibility gene *BRCA1* (breast cancer early onset gene 1) has been routinely sequenced in women with family histories for either malignancy (1). Genetic alterations are reported in a public database (<http://research.nhgri.nih.gov/bic/>), providing a wealth of information on pathogenic mutations in the *BRCA1* gene. Mutations found in either the BRCA1 amino or carboxyl terminus confer highly penetrant breast and ovarian cancer risk, suggesting that each domain within the BRCA1 protein plays an essential role in BRCA1-dependent DNA repair (2, 3), thereby limiting cancer susceptibility. On page 525 of this issue, Shakya *et al.* (4) put this assumption to the test, using elegant in vivo models to show that phosphoprotein binding by the BRCA1 carboxyl-terminal domain is critical for DNA repair and tumor suppression, whereas E3 ligase activity at the amino terminus is not.

The BRCA1 protein is composed of several interaction surfaces, each represented by a specific domain. The first 110 amino acids of BRCA1 comprise the RING domain, the most common structural motif implicated in E3 ubiquitin ligase activity (5) (see the figure). This domain interacts with at least eight different E2 ubiquitin-conjugating enzymes in vitro to affect either mono- or polyubiquitylation of substrate proteins (6). At the opposite end of the protein lie the BRCA1 carboxyl-terminal (BRCT) tandem repeats, a motif that recognizes a phosphorylated serine consensus sequence in at least three different DNA repair protein complexes (7, 8). Pathogenic mutations occur in either domain, suggesting that E3 ligase activity within the RING domain and phosphoprotein interactions at the BRCT repeats both contribute to tumor suppression.

However, many RING domain mutations result in considerable structural perturbation (9), confounding interpretation of whether E3 ubiquitin ligase activity plays an essential role in tumor suppression. To circumvent these concerns, Shakya *et al.* used genetically engineered mice in which isoleucine-26 in the BRCA1 RING domain was replaced with alanine (I26A). This mutation abrogates interaction with E2 enzymes as well as BRCA1 E3 ligase activity, while maintaining overall RING domain architecture (10). The authors also created a BRCT mutation (S1598F, which replaces serine-1598 with phenylalanine) that corresponds to a known cancer-causing allele in humans (S1655F) lacking phosphopeptide recognition. The BRCT mutation accelerated mammary or pancreatic carcinoma formation in three different mouse models. Surprisingly, the RING mutation did not result in any appreciable difference in tumor suppression compared to wild-type BRCA1. The BRCT amino acid change produced genomic instability in cells and tumors, whereas the RING mutation did not in either scenario, highlighting the intimate association of BRCA1 DNA repair and tumor

suppression functions. Moreover, BRCA1 I26A cells did not display any diminution in either homology-directed DNA repair or ubiquitin foci (in response to ionizing radiation) (4, 11), further implying that BRCA1 E3 ligase activity does not play a prominent role in response to DNA damage.

So what purpose does the evolutionarily conserved BRCA1 E3 ligase activity actually serve? BRCA1 I26A mice display small testes, spermatocyte maturation deficits, and male-specific infertility. The molecular basis of these phenotypes is unknown; however, the collective features of BRCA1 I26A cells strongly argue against a general role for BRCA1 E3 ligase activity in meiotic or mitotic recombination. Perhaps a different aspect of BRCA1 biology is in play during male gametogenesis.

BRCA1-dependent ubiquitylation of histone H2A has recently been implicated in transcriptional repression of α -satellite DNA elements (repetitive DNA) embedded within pericentromeric heterochromatin (12). Aberrant expression of α -satellite DNA is a common feature of malignancy (13), and its transcriptional repression, at least in part, requires BRCA1 E3 ligase activity through a putative ubiquitylation of histone H2A. Indeed, BRCA1 I26A cells displayed elevated satellite RNA levels and satellite expression was reduced in BRCA1 mutated cells upon introduction of a histone H2A-ubiquitin fusion protein (12).

As with transcriptional repression of heterochromatin, spermatogenesis necessitates elements of the DNA damage response, including BRCA1 and H2A ubiquitylation, to silence transcription of male sex chromosomes during the pachytene phase of meiotic prophase in a process known as meiotic sex chromosome inactivation (14). These observations warrant investigation of whether BRCA1 E3 ligase activity is a common element for both meiotic sex chromosome inactivation and transcriptional silencing of α -satellite repeats within heterochromatin. Additionally, of interest is whether BRCA1 E3 ligase activity contributes to DNA double-strand break-induced silencing of chromatin in cis to DNA damage sites, because this phenomenon also relies on H2A ubiquitylation (15).

In contrast to the relatively unperturbed DNA repair function in BRCA1 I26A cells, α -satellite RNA overexpression recapitulated loss of BRCA1 DNA damage response activities (12). Ostensibly, increased α -satellite expression in the BRCA1 E3 ligase-deficient mice was not sufficient to incur either genomic instability or cancer. Although the basis for these discrepancies is unclear, it suggests that much remains to be learned regarding how BRCA1 controls genome integrity and tumor suppression. Finally, given the exceedingly complex number of modulatory interactions present in ubiquitin-conjugating networks (16), one cannot definitively discount the possibility that minimal in vivo interaction between BRCA1 I26A and one of the more than 30 different E2-conjugating enzymes could be permissive for a subset of BRCA1 ubiquitylation events. Needless to say, the mysteries of BRCA1 tumor suppression will inspire researchers to continue searching for answers at both ends of the protein.

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Functional ends. Amino-terminal BRCA1 E3 ubiquitin ligase activity is not required for tumor suppression or DNA repair, in contrast to the BRCA1 carboxyl-terminal phosphoprotein binding domain.