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New partners for BRCA1

Inherited mutations in *BRCA* genes predispose individuals to breast, ovarian and other forms of cancer. Although emerging data are helping to define the roles of BRCA proteins and their links to inherited forms of these diseases, much is still unknown about the more prevalent sporadically occurring cancers.

Recently, the *New York Times* told the story of one young woman's decision to undergo a dramatic surgery after receiving the results of a simple genetic test ("Cancer free at 33, but weighing a mastectomy," 16 September 2007). Largely on the basis of maternal family history—three generations of relatives had a startlingly high incidence of breast, ovarian and colon cancers—as well as her mother's experience with breast cancer, she decided to have a preventative mastectomy after learning she carried a mutation in one allele of the *BRCA1* gene. She also expects to undergo a hysterectomy by the age of 40.

The *BRCA1* and *BRCA2* genes are essential to cell growth and development; their deletion is embryonic lethal. Individuals carrying an inherited loss-of-function mutation in the *BRCA* genes are predisposed to the above cancers, indicating that the proteins encoded are tumor suppressors. When coupled to a somatic mutation in its partner allele, an inherited germline mutation in one *BRCA1* or *BRCA2* allele leads to tumorigenesis. Inherited breast cancer accounts for only ~5% of all breast cancers, but since breast cancer is common (1 in 10 women is at risk to develop it), mutations in the *BRCA* genes probably account for a large number of cases.

Both *BRCA* genes were identified more than ten years ago, but their sequences did not initially provide much insight into their function. BRCA1 and BRCA2 have little sequence similarity to each other or to other proteins and both are large proteins, which has not helped characterization efforts. However, BRCA2 was found to have eight copies of an internally repeated 26-residue sequence, called the BRC repeat. BRCA1 was found to have an N-terminal zinc-dependent RING domain, suggesting that it had either DNA-binding or ubiquitin ligase activity. In addition, sequence analysis of BRCA1 revealed repeated motifs at its C terminus, called BRCT repeats, that have since been found in a number of proteins involved in DNA repair. BRCA1 BRCT repeats bind phosphoproteins and mediate protein-protein interactions.

BRCA1 and *BRCA2* mutant cells have defects in DNA repair and suffer from chromosome instability. Although BRCA1 has roles in processes related to these phenotypes, some of which are distinct from that of BRCA2, it is less apparent exactly how it functions in these pathways. Most BRCA1 exists as a heterodimeric complex with BARD1. The BRCA1 RING domain has been shown to function as an E3 ubiquitin ligase that has enhanced activity when coupled with the RING domain of BARD1, and it can be assumed that many of BRCA1's *in vivo* interactions occur in the context of the BRCA1-BARD1 heterodimer. Many cancer-associated mutations in BRCA1 map to its RING domain, where they eliminate ubiquitin ligase activity, as well as to its BRCT repeats.

Much effort has been directed toward linking the enzymatic activity of BRCA1-BARD1 to its role as a tumor suppressor in normal breast and ovarian cells. The ubiquitin ligase activity of the complex is part of an enzymatic cascade that results in the covalent attachment of a ubiquitin moiety to a protein substrate or, in some instances, another ubiquitin molecule. In this cascade, the ubiquitin-activating E1 enzyme transfers an activated ubiquitin to an E2 conjugating enzyme via a thioester intermediate. The charged E2 then interacts with an E3 ubiquitin ligase to specifically target a substrate protein for the covalent modification. For RING E3 ligases, the E3 acts as a scaffolding complex to facilitate direct ubiquitin transfer between the E2 and substrate. The type of ubiquitin linkage formed can be dictated by the E2.

In vitro, BRCA1-BARD1 has been shown to interact with two E2s, UbcH5c and UbcH7, perform autoubiquitination reactions and promote modification of target substrates, but the latter two activities have not been demonstrated directly *in vivo*. Determining the subset of E2s with which an E3 interacts should help identify novel substrates and, in the case of BRCA1-BARD1, help in understanding the enzyme's role in tumor suppression. Klevit and colleagues (page 941 in this issue) have now used a two-hybrid screening strategy, with fused BRCA1-BARD1 RING domains as bait, to identify six new E2 partners—Ubc13-Mms2, Ube2k, UbcH6, Ube2e2, UbcM2 and Ube2w—and have mapped the crucial BRCA1 determinants for these interactions. Of note is the identification of Ubc13, previously implicated in the double-strand break repair pathway. The authors also found that, depending on the E2 present, BRCA1-BARD1 can promote monoubiquitination and specific polyubiquitination chain-forming events *in vitro*, suggesting that specific E2 interactions help target different substrates for different fates.

Showing that a single E3 can promote different ubiquitin conjugation reactions depending on its E2 partner is a significant result, as is the discovery of new E2 partners of BRCA1, which may help in identifying BRCA1 substrates. These findings will pave the way for future studies in both the ubiquitin and BRCA1 fields. However, it is worth noting that, despite an enormous amount of effort thus far, a direct *in vivo* target for BRCA1 activity has yet to be found. Moreover, because BRCA1 seems to function in a network of pathways related to genome stability, identifying which of its effects are related to breast cancer tumor suppression will be complicated. Indeed, as BRCA proteins are part of large multiprotein complexes, many of which include proteins of other cancer-related genes, there may be no clear-cut answer. Even more sobering is the knowledge that 95% of breast cancers occur sporadically, with no mutation in either *BRCA* gene. Certainly, there is much more that remains to be discovered. ■