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NEWS AND COMMENTARY

New breast cancer gene suggests a role for BRCA2 in sporadic cases

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A recent study by Hughes-Davies *et al* [1] found the *EMSY* gene to be amplified in 13% of all breast cancers and 17% of ovarian cancers. EMSY was shown to be capable of silencing the transcription activation potential of a BRCA2 protein domain encoded by exon 3. Like BRCA2, EMSY also relocates to double-strand break repair sites after DNA damage. These results provide a functional link between BRCA2 and EMSY, and imply that BRCA2 inactivation through EMSY amplification might be important in the tumorigenesis of a substantial proportion of noninherited (sporadic) breast cancer.

Approximately 12% of all breast cancer patients have a first-degree relative with breast cancer. Germline mutations in BRCA2 explain about 35% of cases with a strong family history, characterised by multiple early-onset and/or bilateral disease, and an autosomal dominant inheritance pattern. These mutations mostly lead to premature chain termination during protein translation, that is, they are predicted to inactivate the protein. (Many missense changes in BRCA2 have also been found, but the pathogenic potentials of these have remained largely unknown to date.) Most of the breast and ovarian carcinomas developing in BRCA2 mutation carriers show loss of heterozygosity (LOH) at the gene locus involving the wild-type allele.[2,3] These findings are fully compatible with Knudson's 'two-hit' model for the inactivation of a tumor-suppressor gene.[4] LOH at BRCA2 is also detected in about 30–40% of noninherited ('sporadic') breast tumors, but surprisingly few cases have been demonstrated to have inactivated the remaining allele of BRCA2 by a somatically acquired mutation.[5–8] The role of BRCA2 in the genesis of sporadic breast cancer has therefore remained unsettled.[9]

The cellular function of the BRCA2 protein is not precisely known, but current evidence suggests that it is involved in the repair of double-strand DNA breaks by homologous recombination. The middle domain of the 3418 amino-acid protein is characterised by eight repeated motifs, each about 30–40 residues long, that directly interact with the RAD51 protein. RAD51 is a eukaryotic homolog of bacterial RecA and is essential for double-strand break repair. BRCA2 seems to control the formation of RAD51 nucleoprotein filaments that invade homologous DNA duplexes to initiate strand exchange.[10] In addition, the C-terminal third of BRCA2, when in complex with DSS1, a protein of unknown function, can bind single-stranded DNA *in vitro*.[11] BRCA2 is also part of a 2 MDa multiprotein complex that includes BRAF35, an architectural DNA-binding protein capable of binding to cruciform DNA.[12] These interactions point to a role for BRCA2 in chromatin remodelling processes during DNA repair and mitosis. In keeping with this, the N-terminal domain of BRCA2 encoded by exon 3 has been shown to possess histone acyltransferase activity, and has been implicated in the regulation of transcription. Hence, complete loss of BRCA2 function in breast epithelial cells might then equate to loss of maintenance of chromosome stability, fostering further genetic changes leading to cancer.[13,14]

Enter EMSY. This protein was isolated by a yeast two-hybrid screen using the BRCA2 N-terminal domain as bait.[1] EMSY, a novel 1322 amino-acid protein without any homologies to other human proteins, has an exclusively nuclear localization and associates with native BRCA2 *in vivo*. This interaction specifically silences the transactivation activity of BRCA2. In a spontaneously immortalized murine embryonic fibroblast cell line, EMSY also relocates to nuclear foci in response to DNA damage induced by ionising radiation, as does BRCA2. While this suggests some role for EMSY in DNA repair, the authors note that they have not been able to reproduce this result in human cells to date. The N-terminal region of EMSY contains an 80-amino-acid stretch that is evolutionarily conserved. *Arabidopsis* has nine proteins containing such an 'ENT' domain, all of which in turn contain another domain that is part of a superfamily of domains (called the Royal Family) known to recognise methylated lysines within histones. Accordingly, in a yeast two-hybrid assay, EMSY was shown to bind two proteins containing such domains. Taken together, these results imply a role for EMSY in chromatin regulation.

The *EMSY* gene locus maps to chromosome 11q13.5, a region known to be frequently amplified in breast and ovarian cancers. Breast cancer cell lines with such an amplification (five out of 28 examined) always had increased expression of *EMSY* at the mRNA level. The problem with these amplifications is, however, that the amplified genomic region is heterogeneous and usually very large.[15] Several other adjacent candidate genes might

also be 'driving' the amplification, including, in the case of EMSY, *CCND1* (Cyclin D1) and *EMS1*. In one breast cancer cell line, *EMSY* amplification occurred without amplification of *CCND1* or *EMS1*, which was also found for approximately 40% of all primary tumors with *EMSY* amplification. Overall, *EMSY* amplification occurred in 7% of 875 breast tumors analysed by Southern blotting and 13% of 551 cases analysed by FISH. Patients whose tumors had amplified *EMSY* had a worse disease-specific and overall survival, compared to patients without *EMSY* amplification, but this effect was limited to lymph node-negative cases.

Unanswered questions

The interaction of EMSY with proteins that play a role in chromatin remodelling and its ability to bind BRCA2 directly adds weight to a model in which overexpression of EMSY in some sporadic breast tumors is functionally equivalent to loss of the BRCA2 protein as seen in inherited cases. What needs to be done now is to demonstrate that EMSY binds BRCA2 in human mammary epithelial cells – Hughes-Davies *et al* used HeLa cells – and that this interaction leads to suppression of crucial activities of native BRCA2 sufficient to drive tumorigenesis. It is presently unclear whether EMSY targets the transcription activation potential of BRCA2, or its chromatin-remodelling functions. BRCA2 does not contain a Royal Family domain, and it is also not clear how a putative chromatin-remodelling function relates to the known DNA repair activity of BRCA2.

The stoichiometry of the EMSY–BRCA2 interaction is also unknown. Must *EMSY* be amplified in order to suppress BRCA2 function, or, in other words, is *EMSY* amplification an oncogenic event for breast cancer? In this regard, the observed clinical association of EMSY amplification with poor prognosis is not strongly indicative. Although the authors claim that *EMSY* and *CCND1* are independent targets for 11q13 amplification, analysis of the 2×2 contingency table shows that they are strongly related. (If both events were truly independent, one would have expected to see co-amplification in $13\% \times 14\% = 1.8\%$ of all tumors, as opposed to the observed 60%.) Thus, the oncogenic potential of *EMSY* amplification in terms of clinical outcome must be established in the small fraction of breast tumors that have uniquely amplified *EMSY* (3–5% of all breast cancers). It will also be interesting to see if *EMSY*-amplified tumors share any of the morphological and genetic characteristics seen in *BRCA2*-linked breast tumors.[16]

Certainly, this report provides an important new insight into a pathway that might be connected with BRCA2 activity, but further work will be required to understand how its disruption contributes to breast tumorigenesis.

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