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## Breast Cancer Gene Repairs Damaged DNA

Structural studies of the protein produced by the *BRCA2* gene, which is implicated in the development of hereditary breast and ovarian cancers, reveal that the protein is intimately involved in repairing damaged DNA.

DNA-repair proteins perform a vital function and protect against potentially catastrophic events such as cancer-causing mutations or chromosome rearrangements, which are hallmarks of tumor cells.

Howard Hughes Medical Institute investigator Nikola P. Pavletich and his colleagues at Memorial Sloan-Kettering Cancer Center used x-ray crystallography to obtain molecular snapshots of the BRCA2 protein. The images produced by Pavletich's team show that BRCA2 is capable of binding to DNA, a conclusion that is supported by the group's biochemical experiments. The research was published in the September 13, 2002, issue of the journal *Science*.

The scientists were investigating the role of BRCA2 in homologous recombination, which is one of the ways that cells repair broken chromosomes. In this type of DNA repair, broken chromosomes are fixed by using the information from a sister chromosome as a template and splint to guide repair. This type of DNA repair is accurate and is the optimal mode of repair in dividing cells. Prior to the studies by Pavletich and his colleagues, BRCA2 was believed to play only an indirect regulatory role in DNA repair.

BRCA2 had been previously implicated in the control of homologous recombination, although its precise role in this process was unknown, said HHMI investigator Stephen J. Elledge, who is at Baylor College of Medicine. The significance of the structural studies of BRCA2 by Pavletich's group is that they provide evidence for a direct and unexpected biochemical role for BRCA2 in the enzymology of homologous recombination itself. It was an elegant study that will forever change the way we think about BRCA2 and its role in breast cancer. Elledge authored an accompanying article in *Science* that discusses the implications of the findings by Pavletich and his colleagues.

Pavletich's team encountered several major technical hurdles, the first of which involved producing a segment of the BRCA2 protein — called the C-terminal end. We chose this fragment because of evidence — based on its

amino acid sequence, on the fact that it is conserved in many organisms, and on the fact that it is often mutated in tumors — that it carries out an important function in homologous-recombination-mediated repair, said Pavletich.

Producing this large protein fragment of BRCA2 and crystallizing it for x-ray crystallographic studies was a formidable challenge. Lead author Haijuan Yang got a break when she identified a companion protein called DSS1 that bound to the BRCA2 fragment and made it amenable to crystallization.

The scientists then used x-ray crystallography to determine the detailed structure of the BRCA2 fragment. In this process, x-ray beams are directed through purified crystals of a protein, and the resulting patterns of diffraction are analyzed to deduce the proteins structure.

Once we analyzed the structure and compared it with known protein structures, it had domains that looked familiar, said Pavletich. These domains, called oligonucleotide binding folds, are found in proteins known to bind single-stranded DNA.

The scientists then conducted biochemical binding tests *in vitro* using the BRCA2 domain, which revealed that the BRCA2 fragment did indeed bind single-stranded DNA. This finding was confirmed by additional studies in which the researchers crystallized the BRCA2 fragment bound to single-stranded DNA. According to Pavletich, the experiments provided strong evidence that BRCA2 is intimately involved with DNA binding in the repair process.

The researchers discovered that another domain in the BRCA2 fragment binds double-stranded DNA, although they have not yet established conclusively that the domain by itself binds double-stranded DNA.

Pavletichs group also showed that BRCA2 stimulates the activity of an enzyme called RAD51 recombinase, a key component of the DNA-repair machinery. Our observations taken together with other data, suggest that BRCA2 is what recognizes double-strand breaks, said Pavletich. When a cell encounters a double-strand break, it chews up that break to produce single-stranded DNA at the end of double-stranded DNA. And this is what we think is recognized by BRCA2, since it has both single-stranded and double-stranded DNA binding activity.

We were surprised at this direct role of BRCA2, because among scientists in the field, [BRCA2] was thought to be the regulator of RAD51. This function of BRCA2 was more in line with BRCA1, which is thought to be a signaling protein in the process, said Pavletich. Mutations in BRCA1 also have been implicated in the development of breast and other cancers.

Pavletich added that our findings dont reveal any obvious treatment strategies, but as with all basic science, studying how a process works, and how it malfunctions in cancer brings us closer to understanding the process of tumorigenesis. Thus, he said, his laboratory plans detailed studies of how the DNA-binding domain of BRCA2 works in concert with the separate domain

that binds to RAD51, in triggering the DNA-repair machinery.