First purification of cancer gene, BRCA2, studied by UNC scientists

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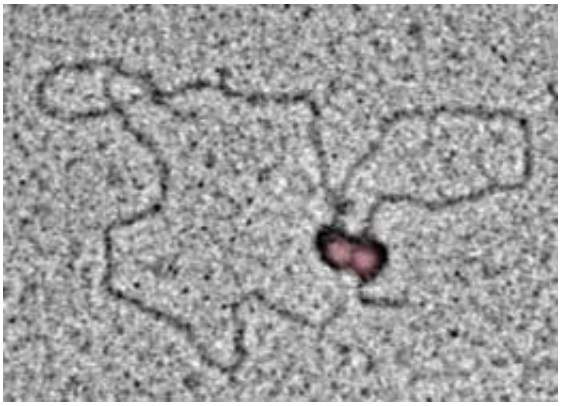

The photo shows two side-by-side molecules of BRCA2 protein, shaded pink, and bound to a circular DNA constructed to contain a binding site for the protein. Image source: Jack Griffith, PhD and Sarah Compton, PhD.

Scientists at the University of North Carolina at Chapel Hill were among co-authors of a study that described the first isolation and purification of the BRCA2 protein which is produced by a gene whose loss greatly increases the risk of developing breast and ovarian cancers.

In a report of the findings published online by the journal Nature Structural & Molecular Biology on Aug. 22, 2010, Sarah Compton, PhD and Jack Griffith, PhD of UNC Lineberger Comprehensive Cancer Center joined study leader Stephen West of the London Research Institute and other co-authors. Their findings could lead to a better

understanding of how the protein works and how BRCA2 sequence mutations cause cancer.

The protein has been notoriously difficult to isolate until now. As one of the largest proteins in a cell - eight to ten times larger than the average protein size - it can't be expressed in bacteria in order to be isolated like other proteins.

The accomplishment may also open a door to the development of new cancer therapies that could block the cancer-causing process. Knowledge of a protein and how it behaves could lead to the development of a search for chemical compounds, and eventually drugs that can stop mutant versions of the protein from wreaking havoc in cells.

Three laboratories independently accomplished the feat. Their three separate papers were published online Aug. 22, one by the journal *Nature* and two by the journal *Nature Structural & Molecular Biology*.

According to a summary of these efforts in Nature News, the three studies explored the interaction of BRCA2 protein with other proteins, primarily one called RAD51. This protein repairs DNA by assembling around breaks in DNA strands, and forming filaments through which DNA components called nucleotides are pulled in to fix gaps. By studying the interaction between BRCA2 and RAD51, all three teams confirmed that BRCA2 helps RAD51 initiate filament growth.

Griffith is an internationally renowned DNA electron microscopist. The Kenan Distinguished professor's electron microscopy (EM) work includes a number of breakthroughs, beginning in his graduate school years. For his PhD work at Cal Tech, Griffith developed the EM technology needed to directly visualize bare DNA and DNA-protein complexes. His methods involved carefully controlled rotary shadow casting with tungsten and mounting the DNA on very thin carbon films.

Using the methods he developed, Griffith, with Drs. Jack Kornberg and Joel A. Huberman, published a paper that carried the first EM image of DNA bound to a known protein. It also showed that electron microscopy had the potential to provide quantitative information about macromolecular assemblies involving DNA.

And in 2002, Griffith and colleagues used quantitative techniques to map the DNA involved in Fragile X syndrome. In people with Fragile X, a particular DNA sequence is repeated too often — as many as two thousand times, compared to only seventeen to thirty times in normal DNA. But it wasn't known how that repetition, called expansion, contributed to Fragile X syndrome. "We showed that in Fragile X, that expansion creates a segment of the chromosome that is very unorganized and unprotected relative to the rest of the chromosome," Griffith told Endeavors, UNC's research magazine. The work provides a clue to the molecular causes of the disorder.

Provided by University of North Carolina at Chapel Hill School of Medicine