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Protein made by breast cancer gene purified

23.08.2010

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A key step in understanding the origins of familial breast cancer has been made by two teams of scientists at the University of California, Davis. The researchers have purified, for the first time, the protein produced by the breast cancer susceptibility gene BRCA2 and used it to study the oncogene's role in DNA repair.

The results will be published online Aug. 22 in the journals Nature, and Nature Structural and Molecular Biology. They open new possibilities for understanding, diagnosing and perhaps treating breast cancer.

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BRCA2 is known to be involved in repairing damaged DNA, but exactly how it works with other molecules to repair DNA has been unclear, said Stephen Kowalczykowski, distinguished professor of microbiology in the UC Davis College of Biological Sciences, UC Davis Cancer Center member and senior author of the Nature paper.

"Having the purified protein makes possible far more detailed studies of how it works," Kowalczykowski said.

Kowalczykowski's group has purified the protein from human cells; another group led by Professor Wolf-Dietrich Heyer, also in the UC Davis Department of Microbiology and leader of the Cancer Center's molecular oncology program, used genetic engineering techniques to manufacture the human protein in yeast. That work is published in Nature Structural and Molecular Biology.

The two approaches are complementary, Heyer said, and the two teams have been talking and cooperating throughout.

"It's nice to be able to compare the two and see no disagreements between the results," Heyer said.

Experiments with the BRCA2 protein confirm that it plays a role in repairing damaged DNA. It acts as a mediator, helping another protein, RAD51, to associate with a single strand of DNA and stimulating its activity. One BRCA2 molecule can bind up to six molecules of RAD51.

The RAD51/DNA complex then looks for the matching strand of DNA from the other chromosome to make an exact repair.

If the BRCA2/RAD51 DNA repair system is not working, the cell resorts to other, more error-prone methods.

"It's at the apex of the regulatory scheme of DNA repair," Kowalczykowski said. Your DNA is constantly suffering damage, even if you avoid exposure to

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carcinogens. If that damage is not repaired, errors start to accumulate, Kowalczykowski said. Those errors can eventually lead to cancer.

The BRCA2 gene was discovered in 1994. Mutations in BRCA2 are associated with about half of all cases of familial breast and ovarian cancer (cases where the propensity to develop cancer seems to be hereditary), and are the basis for genetic tests.

But purifying the protein made by the gene has proved difficult.

"It's very large, it does not express well, and it degrades easily," Kowalczykowski said.

Ryan Jensen, a postdoctoral researcher in Kowalczykowski's lab, after testing many different cell lines, succeeded in introducing a BRCA2 gene into a human cell line and expressing (producing) it as a whole protein. Jensen and another postdoc, Aura Carreira, tested the purified protein for its function in repairing damaged DNA.

Jie Liu, a postdoctoral researcher in Heyer's lab, found that a much smaller protein called DSS1 stimulated BRCA2 to assemble functional RAD51/DNA complexes. Together with Liu, staff research associate Tammy Doty and UC Davis undergraduate student Bryan Gibson (now a doctoral student at Cornell University) purified the human BRCA2 and DSS1 proteins from yeast.

One application of the purified protein would be to make antibodies to BRCA2 that could be used in test kits as a supplement to existing genetic tests, Kowalczykowski said.

A more exciting possibility, he said, would be to use the system to screen for drugs that activate or inhibit the interaction between BRCA2, RAD51 and DNA. Many cancer treatments work by creating breaks in DNA, and a drug that selectively shuts down a specific DNA repair pathway -- making it harder for cancer cells to recover -- could make the cells more vulnerable to treatment. That strategy is already being exploited by a new class of drugs called PARP inhibitors, currently in clinical trials. PARP inhibitors target an alternate DNA repair pathway that cells use when the BRCA2 repair pathway is not available.

The BRCA2 protein can also be used to study how different mutations affect the gene's function.

"We're just starting to scratch the surface and understand more of the mechanisms and interaction with other factors," Kowalczykowski said.

The work was supported by grants from the National Institutes of Health, the U.S. Department of Defense Breast Cancer Research Program, the Susan G. Komen Breast Cancer Foundation, and the UC Davis Cancer Center. Jensen was supported by a fellowship from the American Cancer Society; Carreira was supported by a fellowship from the Spanish Ministry of Education and Science, and Liu by a fellowship from the Tobacco-Related Disease Research Program.

About the UC Davis Cancer Center

UC Davis Cancer Center is the only National Cancer Institute-designated center serving the Central Valley and inland Northern California, a region of more than 6 million people. Its specialists provide compassionate, comprehensive care for more than 9,000 adults and children every year, and offer patients access to more than 150 clinical trials at any given time. Its innovative research program includes more than 280 scientists at UC Davis and Lawrence Livermore National Laboratory. The unique partnership, the first between a major cancer center and national laboratory, has resulted in the discovery of new tools to diagnose and treat cancer. For more information, visit www.ucdmc.ucdavis.edu/cancer.

About the College of Biological Sciences

The Department of Microbiology is one of five departments in the UC Davis College of Biological Sciences, one of few colleges in the country dedicated entirely to the study of basic biology. The college's faculty, researchers and students are advancing the planet's knowledge on many frontiers by exploring fundamental questions about life.

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