

BRCA2, Purified, Reveals Some of Its Secrets

By Charlie Schmidt

Working independently, three research teams have purified extracts of human BRCA2 protein, encoded by a tumor suppressor gene that, when mutated, sharply elevates risks for breast and ovarian cancer. The achievement lends new insights into BRCA2 functioning and could lead to new cancer treatments,

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according to Gordon Mills, M.D., Ph.D., chair of molecular oncology at the M. D. Anderson Cancer Center in Houston.

“I’d describe it as a technical tour de force,” said Mills, who was not involved in the work. “It refines our understanding of the mechanism by which BRCA2 coordinates DNA repair, and it could have a massive impact on developing new ways to go after abnormalities in both BRCA1 and BRCA2.” The results were published online in *Nature* and *Nature Structural Biology* in August.

BRCA2 ranks among the largest human proteins, with 3,418 amino acids. Scientists struggled for 15 years to purify the giant molecule, which tends to break into fragments almost as soon as it’s transcribed in the laboratory. To get around this problem, researchers had to affix protein tags to BRCA2’s front and back ends, which held the molecule in place during extraction from cell-based expression systems. Remarkably, each research team purified BRCA2 around the same time, though each used different tags and different systems.

Stephen C. Kowalczykowski, Ph.D., a biochemist at the University of California, Davis, who led the team that published in *Nature*, said that hitting on the right combination of tags and expression systems took

considerable trial and error. Neither bacterial nor insect cell lines possessed the protein mix that human BRCA2 relies on to fold correctly as it emerges from the genome, he said. Kowalczykowski’s postdoctoral student, Ryan Jensen, Ph.D., ultimately succeeded by using human cells and tags made of maltose binding protein, which attach preferentially to amylose resins used in chromatography columns. Like magnets on a refrigerator door, these proteins hold BRCA2 fast during the extraction, trapping the molecule while other proteins in the cell slip by.

Wolf-Dietrich Heyer, Ph.D., also a biochemist at UC Davis, and lead author on one of the *Nature Structural Biology* reports, successfully purified BRCA2 in yeast, using a glutathione S-transferase tag on the protein’s N terminus (front end) and a histidine affinity tag on its C terminus (back end). “We used yeast because we’ve got a lot of experience with it,” Heyer said. “We had a lot of tricks we could use to express the protein and also to preserve it.”

Finally, Stephen West, Ph.D., a geneticist at Cancer Research UK in London, and colleagues, who also published in *Nature Structural Biology*, purified BRCA2 in cultured human HeLa cells made in a bacterial construct.

While acknowledging the purification’s importance to science, Kowalczykowski also describes the achievement as more of a means to an end. “The more important story is what we can do with the full-length validated protein in hand,” he said. “Now we can run detailed mechanistic and molecular studies to explore what BRCA2 does.”



Stephen C. Kowalczykowski, Ph.D.

Scientists previously inferred BRCA2's behavior by using fragments of the molecule, or "BRCA-like" proteins, that fungi and other simple organisms produced. These earlier studies showed that BRCA2's main role is to coordinate homologous recombination, a precise method for DNA repair.

RAD51 Role

The new findings expand on what's known about this process. According to the results, BRCA2 regulates homologous recombination by interacting with a second

protein, RAD51, which scours the genome for undamaged DNA sequences that can serve as genetic patches. Every gene has an identical (or homologous) partner in the genome, and it's unlikely that both members will sustain the same damage. Each BRCA2 binds to six RAD51 proteins, the new research shows. The RAD51 then forms filaments on damaged DNA and searches for a homologous sequence. On finding that sequence, RAD51 transfers replacement DNA building blocks back to the damaged site for repair, Heyer said.

Many features of this process remain unclear. But according to the new research, BRCA2 instigates RAD51 in part by displacing a competing molecule—replication protein A—which binds DNA and triggers other, less effective repair mechanisms. "The essential idea is that BRCA2 integrates all the information cells need to decide what type of DNA repair they're going to use, or if they're not going to repair themselves at all, or if they're going to undergo programmed cell death," Heyer said. "That's what makes BRCA2 such a prominent tumor suppressor."

If patients lose both their BRCA2 genes to inherited and epigenetic mutations, homologous recombination fails. Cells then revert to a more error-prone process, excision repair, which leads to an accumulation of DNA damage and an approximately 90% risk for either breast or ovarian cancer. Scientists still don't know why BRCA2 mutations boost breast and ovarian cancer risks so much more than risks for other malignancies. "That's the \$64,000 question," Powell said.

Next Steps

Further studies with the new protein could go a long way towards exposing how BRCA2 functions in recombinational repair and cancer biology. West said that he plans to investigate the protein's biochemistry, particularly its interactions with RAD51 and replication protein A.

"And there's a network of other proteins that also work with BRCA2 in DNA repair, and if you disrupt any one of them it's likely that cancer will ensue," Kowalczykowski said.

"Right now, we only understand those interactions at a superficial level." Other experts said that scientists will now be able to introduce new mutations in the BRCA2 sequence and then purify the altered protein to study how those alterations affect its function.

One limiting factor, however, is scaleup—current methods allow purifying BRCA2 only in microgram quantities. These quantities are sufficient for biochemical studies, said West, who added that he was impressed by how little BRCA2 is needed to study interactions with other proteins. But he conceded that neither he nor other researchers can now make enough pure protein for structural studies—such as 3-D nuclear magnetic resonance spectroscopy—that might reveal pharmacological opportunities to restore BRCA2 function.

Scientists are now working to treat BRCA1 and BRCA2 cancers by blocking excision repair, which is how cancer cells fix their own DNA when homologous recombination fails. That's the strategy behind PARP [poly(ADP-ribose) polymerase] inhibitors,

experimental drugs that inhibit PARP, an enzyme required for excision repair. These drugs have shown promise in clinical trials targeted at BRCA1- and BRCA2-positive breast, ovarian, and prostate malignancies. Perhaps a future generation of drugs, Kowalczykowski said, could treat cancer by enhancing BRCA2 activity so that it doesn't lose all ability to interact with RAD51, for instance.

According to Mills, the purification was an important step toward scaling up to make enough BRCA2 protein for structural research. "BRCA2 is large, and it's also relatively unstable," he said.

"That's a real problem because you need a stable protein to develop crystal structures to analyze. But before you can even think of attempting this, you need the pure protein, which no one was able to make previously, even in limited quantities. So this is an important contribution."

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