# Advancing breast, ovarian cancer research with cryo-electron microscopy

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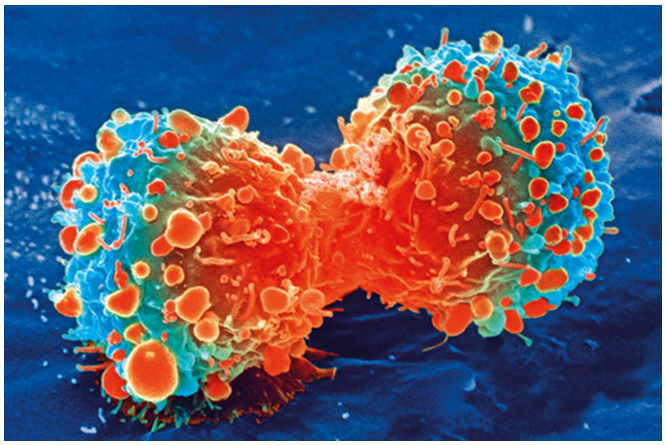

Cancer cell during cell division. Credit: National Institutes of Health

Using advanced imaging technology, Mayo Clinic scientists have provided an unprecedented understanding of the BRCA1-BARD1 protein complex, which is often mutated in patients with breast or ovarian cancer. Their paper, published in *Nature*, identifies aspects of how BRCA1-BARD1 functions, supporting future translational research, cancer prevention efforts and drug development.

"BRCA1-BARD1 is important for DNA repair. It has direct relevance to cancer because hundreds of mutations in the BRCA1 and BARD1 genes have been identified in cancer patients," says Georges Mer, Ph.D., a Mayo Clinic structural biologist and biochemist who is the lead author of the paper. "But no one knows if these mutations, or variants of unknown significance, are cancer-predisposing or not because we do not know whether the variants are located in a region of BRCA1-BARD1 that is important for function. Now because we can see how BRCA1-BARD1 works, we have a good idea of what regions of BRCA1-BARD1 are important for function."

In a cell, the complex of DNA and histone proteins are complexed into what's called chromatin, and packaged into bundles called nucleosomes. DNA damage response proteins need to access chromatin to repair damaged DNA. BRCA1-BARD1 contributes to fixing broken DNA strands, which helps in the maintenance and survival of cells. But it is also a function that could possibly be blocked or inactivated if this is a strategy a cancer cell uses to survive chemotherapy.

**Cryo-electron microscopy and nuclear magnetic resonance spectroscopy**

"We used two techniques?cryo-electron microscopy and nuclear magnetic resonance spectroscopy?to understand at near-atomic resolution how BRCA1-BARD1 associates with the nucleosome, the repeating unit of chromatin, and how BRCA1-BARD1 modifies chromatin," explains Dr. Mer.

In cryo-electron microscopy, purified BRCA1-BARD1 bound to nucleosomes, together referred to as macromolecules, are flash-frozen then imaged using an electron microscope. The macromolecules are oriented in various ways within the sample so a computer program evaluates all the orientation data to create a 3D structure. Dr. Mer and his team also examined BRCA1-BARD1 nucleosome complexes with nuclear magnetic resonance spectroscopy, which uses a strong magnet to probe the relative positions of atoms within macromolecules. Using these imaging tools, the scientists could visualize BRCA1-BARD1 in action and uncover a new function of the complex.

"We showed how BRCA1-BARD1 attaches ubiquitin to the nucleosome, but we also determined that BRCA1-BARD1 recognizes ubiquitin already attached to the nucleosome, which serves as a signal for broken DNA," says Dr. Mer. "We discovered an unexpected cross-talk by which ubiquitin recognition by BRCA1-BARD1

enhances its ubiquitin attachment activity, and this helps us better understand how BRCA1-BARD1 performs its function."

The researchers created a video from the cryo-electron microscopy data to show where the protein complex interacts with the nucleosome.

**From discovery science to patient care**

Dr. Mer and his team expect that high-resolution images of BRCA1-BARD1 can help guide patient care and future treatment of cancer in two ways: classifying variants of unknown significance and directing drug development with more accuracy.

"With these 3D structures, we should be able to convert several variants of unknown significance to likely cancer-predisposing variants," says Dr. Mer. "This work is also expected to have an impact on drug development in the long term because the 3D structures of BRCA1-BARD1 in complex with the nucleosome we generated may help in the design of small molecules that could, for example, inactivate BRCA1-BARD1."

In addition to Dr. Mer, other authors on the paper are Qi Hu, Ph.D.; Maria Victoria Botuyan, Ph.D.; Debiao Zhao, Ph.D.; Gaofeng Cui, Ph.D.; and Elie Mer. This research was funded by the National Institutes of Health, Mayo Clinic Cancer Center, Mayo Clinic Center for Biomedical Discovery, and the Ovarian Cancer Research Alliance, and was made possible through cryo-electron microscopy and nuclear magnetic resonance instrumentation at the Pacific Northwest Center for Cryo-EM and Mayo Clinic, respectively.

**More information:** Qi Hu et al, Mechanisms of BRCA1–BARD1 nucleosome recognition and ubiquitylation, *Nature* (2021). DOI: 10.1038/s41586-021-03716-8