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Blood relations: New study explores early detection of ovarian cancer

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Despite many research advances, ovarian cancer remains lethal in a majority of cases, due to late diagnosis of the disease. In a new study, Dr. Joshua LaBaer of the Biodesign Institute at Arizona State University, along with Arturo Ramirez and Paul Lampe, researchers at the Fred Hutchinson Cancer Research Center in Seattle, used a novel method for identifying biomarkers—proteins in blood that can identify ovarian cancer before symptoms appear.

The work, which appeared recently in the journal *Molecular and Cellular Proteomics*, holds the potential for significant improvements in patient survival rate. The research is part of the Early Detection Research Network program of the National Cancer Institute.

As LaBaer notes, ovarian cancer is an attractive target for biomarker study. "This is a disease for which an early diagnostic test would make an enormous difference in the health of women." Highly treatable in its early stage, ovarian cancer is typically not identified until it has progressed to stage 3 or beyond. Often, it is detected accidentally, in the course of some other test or procedure, for example, during an oophorectomy. "By the time it's caught," LaBaer says, "it has usually speckled the abdomen with advanced tumors."

At present, only one reliable biomarker for ovarian cancer exists. Known as CA 125, this protein is produced on the surface of cells and released into the bloodstream. Elevated levels of CA 125 are indicative of ovarian cancer, but testing for CA 125 alone is not adequate. Such tests can

produce both false positive and false negative results. Further, the level of CA 125 tends to go up in proportion to tumor growth, sometimes providing strong evidence only after the disease has reached its later, terminal stages.

LaBaer stresses that reliable early detection will require the discovery and combined application of multiple biomarkers. One innovative way to hunt for them involves the use of antibodies. These proteins—produced by the B cells of the body's immune system—are able to selectively bind with disease-associated antigens. Various techniques permit researchers to probe blood serum, pulling out the antigens that bind to the antibodies and observing them with the aid of fluorescence or other methods.

Traditional means of producing antibodies for research are labor-intensive and cumbersome, requiring injection of antigens into animals, which then act as production sites for the antibodies of interest. A new method however allows antibodies to be engineered synthetically. In this scenario, the portion of the antibody responsible for binding with an antigen, known as the variable region, is built from amino acids. These single chain variable fragments or scFvs can be inserted into bacteria, which act as vectors for the antibody fragments, much as a mouse or other animal would carry a traditional antibody.

scFvs encoded in bacteria have been assembled into vast libraries, capable of probing the full complexity of proteins found in blood. When an scFv detects something unusual in the blood that may suggest an abnormality, it binds to it, just as in a normal immune response. Ramirez used a library of scFvs to find which antibody fragments selectively attached to proteins in blood carrying ovarian cancer, eliminating the antibodies that bound with proteins in normal blood. The method yielded 19 distinct scFvs that appeared to display specific affinity for proteins exclusively found in ovarian cancerous blood serum.

Identification of the protein antigens bound to these scFvs however, proved far trickier. After several years of frustrating attempts to recognize these protein culprits, Ramirez teamed up with LaBaer, applying a new method that would allow them to screen the cancer-associated scFvs against thousands of proteins of known sequence.

The technique—known as NAPPA (for nucleic acid programmable protein array), provides a convenient means to display thousands of different proteins. Further, proteins used in NAPPA are synthesized freshly for each experiment and do not require the lengthy processes of purification necessary with conventional protein arrays.

When the team screened the 19 selected ScFvs, they found that about two thirds stuck to a target in the NAPPA array, allowing for positive identification. Intriguingly, these distinct ScFvs bound with the same protein, evidentially attaching to different regions. As LaBaer notes, this fact offers tantalizing hints that the technique may indeed have sniffed out a consistent biomarker for ovarian cancer, rather than anomalous proteins lacking true predictive power.

The study also examined proximal tumor fluid for ovarian cancer, detecting in high concentrations the key protein antigens identified with the microarray. According to LaBaer, this finding provides further support for the idea that these proteins are indeed associated with ovarian cancer, and do not represent mere anomalies.

Ramirez and Lampe plan follow-up work to further validate the proteins discovered and assess their clinical value as early detectors of ovarian cancer. They also have protein candidates for other cancers and hope to screen these as potential biomarkers, using LaBaer's NAPPA technology.