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A new toolkit for studying how 'PARP' activity boosts cancers

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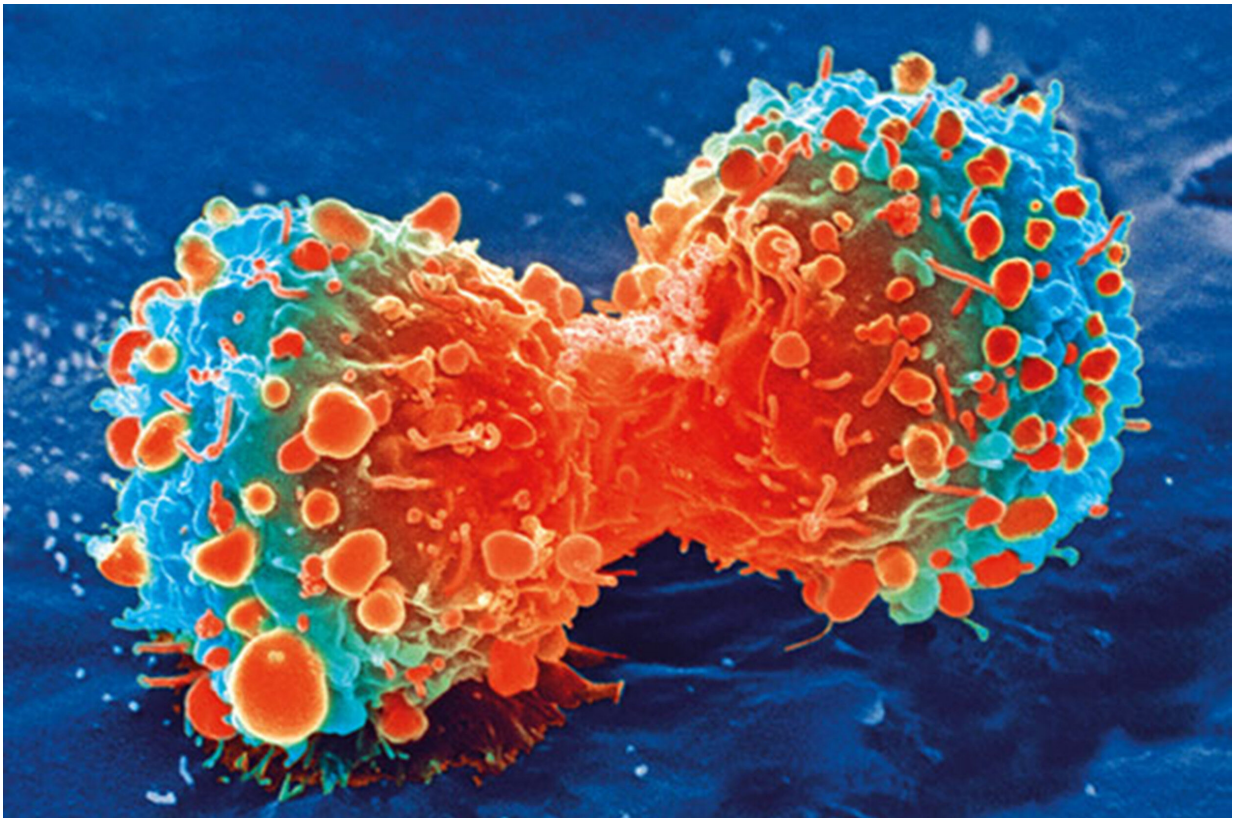

Cancer cell during cell division. Credit: National Institutes of Health

A new method developed by scientists at Johns Hopkins Bloomberg School of Public Health is likely to speed the study of an important biological process called ADP-ribosylation.

ADP-ribosylation occurs at high levels in some cancers, and drugs called PARP inhibitors, which block ADP-ribosylation, comprise one of the most promising new classes of cancer therapy. Yet research in this important area has been limited by the lack of laboratory methods for studying ADP-ribosylation. PARP stands for poly ADP ribose polymerase.

The new method, described in a paper that appears Jan. 31 in *Molecular Cell*, enables scientists to fasten tiny beacons or chemical handles onto ADP-ribose molecules in order to study in detail how ADP-ribosylation affects cells in health and disease.

"The technique we've developed is simple, versatile and most importantly, can be done by any cellular or molecular biology lab," says senior author of the study, Anthony K. L. Leung, Ph.D., an associate professor at the Bloomberg School in the Department of Biochemistry and Molecular Biology. "Our technique also saves time—one to two hours versus more than two days using the previous technique."

ADP-ribosylation is a process that involves the attachment of one or more ADP-ribose molecules to a protein. It is among several reversible chemical modifications that cells commonly use to fine tune the activities of different proteins, and it is known to support a variety of important cellular processes, including the repair of damaged DNA.

PARP inhibitors are designed to target cancers that already have existing defects in DNA repairs. As a result, these cancers rely on the remnant DNA repair pathways mediated by PARP for survival. By inhibiting these PARP-mediated remnant pathways, cancer cells can be killed, while sparing healthy cells with functional DNA repair. PARP-inhibitor drugs are now used to treat patients with the infamous BRCA "hereditary" genes in ovarian, breast and other cancers.

Scientists in recent years have found evidence suggesting that excess ADP-ribosylation also contributes to Parkinson's disease, Alzheimer's disease and other forms of neurodegeneration. A study from Leung and colleagues' laboratories in 2017 found evidence that some dangerous viruses reverse ADP-ribosylation to facilitate infection.

Previously, researchers had only limited and relatively cumbersome techniques for studying ADP-ribosylation in the laboratory. In particular, the traditional, organic-chemistry-based method for attaching molecular beacons or chemical handles to ADP-ribose requires a complex setup, takes days to accomplish, works with low efficiency and works only on "free" ADP-ribose molecules as opposed to ADP-ribose that is bound to one of its protein targets.

In the new study, Leung and colleagues demonstrated that they could use an enzyme called oligoadenylate synthetase 1 to efficiently attach a molecule called dATP to one end of ADP-ribose, whether free or protein-bound. They showed that they could attach not only plain dATP but also variants or analogs of dATP that incorporate fluorescent or radioactive atoms—beacons that are useful, for example, in studying how ADP-ribosylation affects the functions of the proteins it modifies.

The scientists also found that they could attach dATP analogs that work as chemical handles, enabling the isolation of even trace quantities of ADP-ribosylated proteins from a solution in order to identify the proteins that have been modified this way.

ADP-ribose molecules often attach to proteins not singly but in chain-like units or polymers known as poly ADP ribose (PAR). Leung and colleagues showed that their new technique can be used to gauge the lengths of these PAR polymers on the proteins to which they have attached. The new method thus enables the study of how PAR polymer length determines the effect of ADP-ribosylation on any given protein.

In principle, the same approach could be used to evaluate how well PARP inhibitors reduce PAR polymer length on key proteins, and how that length-reduction correlates with cancer outcomes.

The scientists have dubbed their new technique, "ELTA"—Enzymatic Labeling of Terminal ADP-ribose.

"You can think of ELTA as an adapter for existing molecular biology techniques to study the PAR polymer," Leung says.

Leung and his colleagues expect that ELTA will be widely used in the study of ADP-ribosylation and related processes—both for pure biology and for research aimed at clinical applications. "It should enable new diagnostic methods that would use PAR polymer length as a biomarker for cancers and other diseases," Leung says.

More information: "ELTA: Enzymatic Labeling of Terminal ADP-ribose"*Molecular Cell* (2019).

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