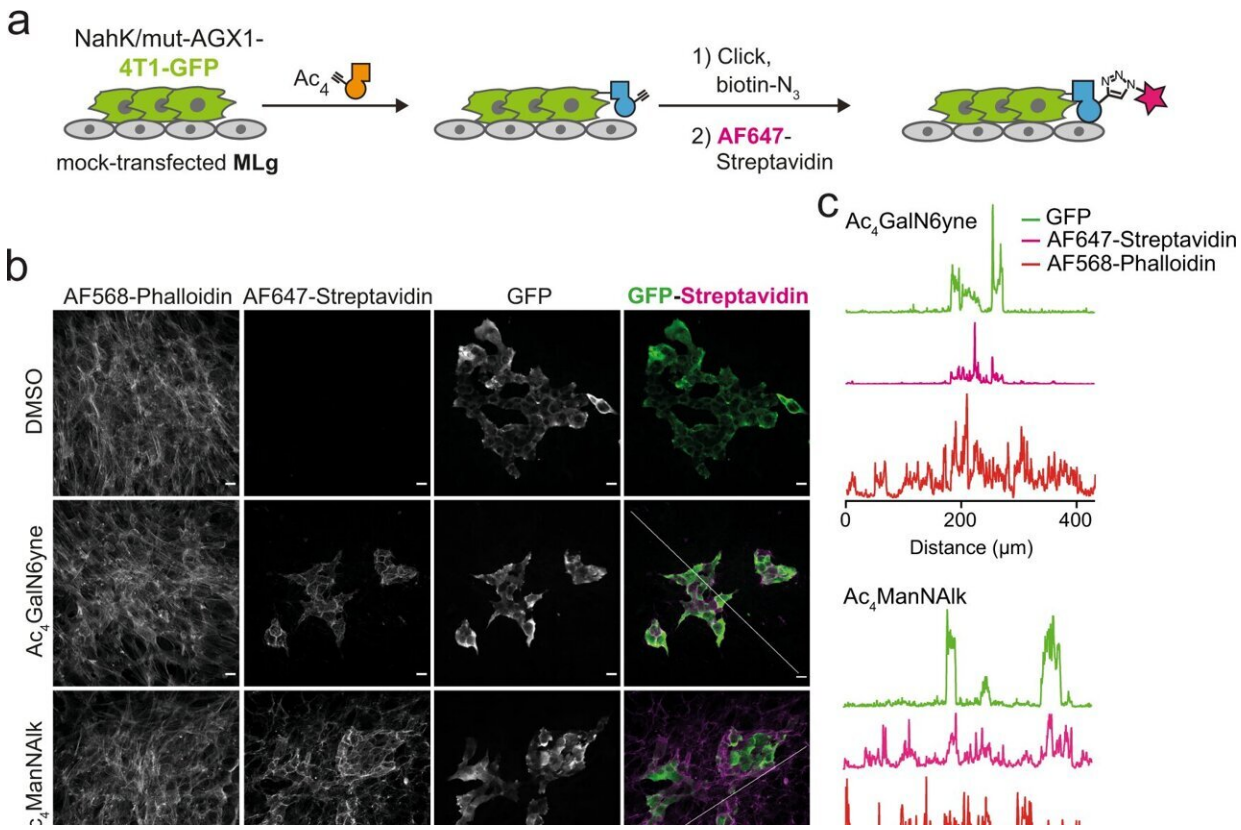
New method to label proteins could help track disease

October 25 2022



Bioorthogonal cell-specific glycoprotein tagging in co-culture. a Schematic of the 4T1-MLg co-culture experiment. Green fluorescent protein (GFP)-expressing 4T1 cells transfected with NahK/mut-AGX1 should be selectively positive for AlexaFluor647-labeling in BOCTAG. b Fluorescence microscopy, using co-cultures fed with 50 µM Ac_4GalN6yne or 50 µM Ac_4ManNAlk as well as AlexaFluor568-Phalloidin as a counterstain. Scale bar, 20 µm. c Intensity profiles of fluorescent signal between GFP and AF647 in Ac_4GalN6yne- (top) or Ac_4ManNAlk-fed (bottom) co-cultures. The intensity

profiles of GFP, AF647-Streptavidin and AF568-Phalloidin signals were measured along a diagonal line drawn across the fluorescent image. Credit: *Nature Communications* (2022). DOI: 10.1038/s41467-022-33854-0

A new method to study the proteins released by cells, which could lead to the development of new tools to track diseases including cancer, has been developed by scientists at the Francis Crick Institute and Imperial College London.

Biomarkers are highly valuable tools that allow doctors to study biology and disease, for example, diagnose a disease from a blood or tissue sample, predict if a treatment will be effective in an individual or see how much of a drug is reaching diseased cells.

But finding these biomarkers is challenging. To help diagnose disease, scientists need to identify proteins that are uniquely made by diseased or cancerous cells but are not released by healthy cells.

In their study, published in *Nature Communications* today (October 25) the team developed a new method that identifies proteins released by a specific type of cell, even if the cells are in a complex environment with lots of other cell types.

"When you have a sample containing various cell lines, it is very difficult to identify the proteins that came from a specific line. Of course, in the laboratory, we can create experiments with only one type of cell, however these conditions do not mirror what happens in the body where complex interactions between cells could affect their behavior and so the proteins they release," explains Ben Schumann, lead author and group leader at the Crick and Imperial College London.

The new method is centered around adding chemical tags to sugar molecules which are added to cells. While all cells absorb the sugar, the researchers genetically modify the cell type they want to study, so that only this type adds the sugar to its proteins. When the cells make these proteins, they remain marked with the chemical tag, meaning that researchers can identify them.

The method uses bioorthogonal or "click" chemistry which was awarded this year's Nobel Prize in Chemistry. One of the prize recipients, Carbolyn Bertozzi from Stanford University, is a co-author of this study. The chemical tag is selected so that it "clicks" with another molecule that helps the researchers isolate the desired proteins or add a fluorescent tag to them.

The researchers showed their method, called Bio-Orthogonal Cell line-specific Tagging of Glycoproteins (BOCTAG), worked in cell cultures with multiple cell lines and also in mice, where the researchers successfully tagged proteins from particular cancer cells.

"In this study, we looked at proteins made by cancer cells, but our method could also be used in other fields including immunology or the study of infectious disease. It could also be used to better understand disease biology, including how tumor cells change as a result of complex interactions in the body," says Anna Cioce, first author and postdoctoral training fellow at the Crick.

"The next step for our team will be to continue developing this method and learning more about how cells produce different proteins depending on their environment," adds Schumann.

More information: Anna Cioce et al, Cell-specific bioorthogonal tagging of glycoproteins, *Nature Communications* (2022). DOI: 10.1038/s41467-022-33854-0

Provided by The Francis Crick Institute