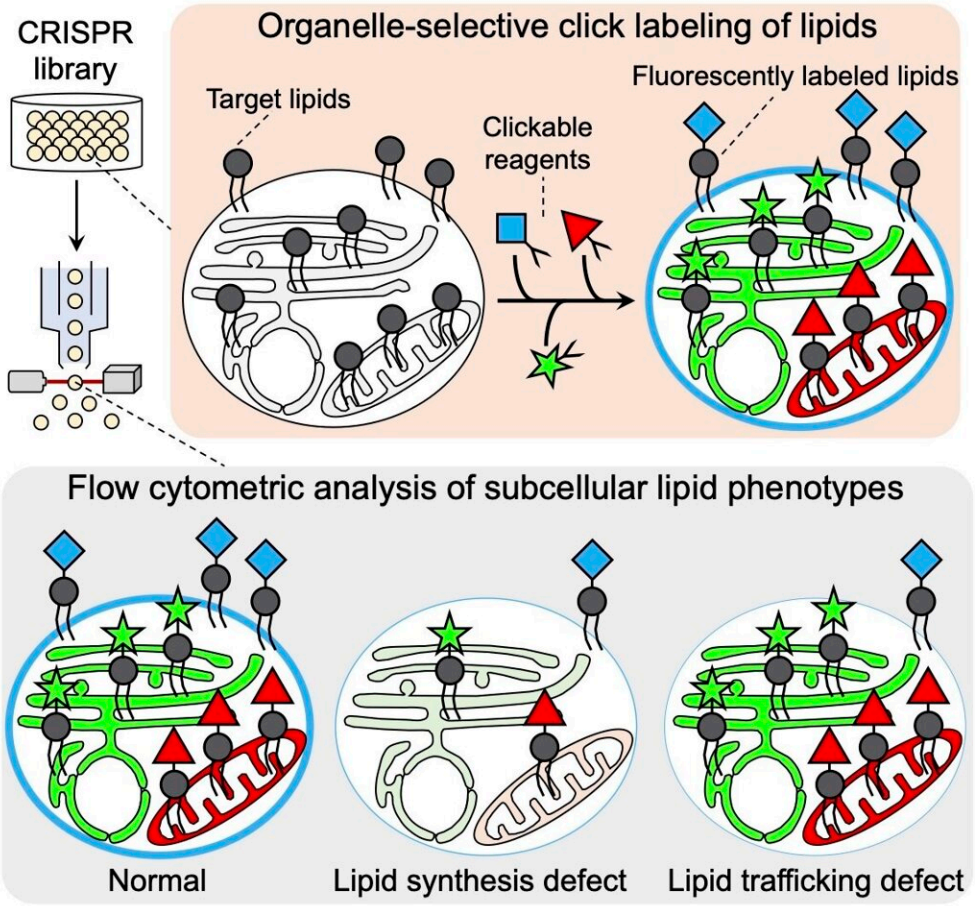
Novel click chemistry technology for ultrafast analysis of intracellular lipids

March 27 2023



Target lipids in live cells are fluorescently labeled via spatially limited click reactions using different-colored organelle-targeting reagents. This allows for flow cytometric high-throughput analysis of their lipid contents and distribution at the organelle level on the basis of fluorescent intensity and spectrum of labeled lipids. Fluorescent patterns on labeled lipids in individual cells reflect the metabolic status such as defects in lipid biosynthesis or trafficking caused by

CRISPR gene editing, thereby enabling large-scale genetic screening based on subcellular lipid phenotypes. Credit: JST

Conventional analysis of cellular lipids mainly involves radiometric analysis and mass spectrometry of cell extracts collected in large quantities. This method is time-consuming and labor-intensive and burdensome when analyzing a large number of samples. The issue needs to be addressed to elucidate the relationship between 20,000 types of human genes and lipid metabolism.

In a new study, researchers from Kyoto University have developed O-ClickFC, a technology that converts the abundance and spatial distribution of lipids in living cells into simple fluorescent signal information and analyzes them at an ultra-high speed (10,000 cells per second). This is done using a unique click reaction that can label lipids with fluorescent dyes in living cells. By combining this technology with "genome editing," it is possible to select cells with abnormal lipid metabolism from a cell population with mutations in all human genes and identify the causative genes.

As a demonstrative experiment, the research group identified 49 genes important for the metabolism of phosphatidylcholine (PC), a major component of human lipids, and discovered many novel genes, including FLVCR1, in the process. From a thorough analysis, it was found that FLVCR1 played a role in the uptake of choline, a nutrient essential for normal bodily functions and human health. Furthermore, the researchers elucidated part of the pathogenesis mechanism in mutant FLVCR1, which causes hereditary neurological disease and loss of choline uptake.

The research is published in the journal *Cell Metabolism*.

It has become evident over the years that metabolic abnormalities are the source of diseases such as cancer, obesity, and diabetes. O-ClickFC may be thereby applied for the analysis of not only lipids but also various metabolites such as sugars and amino acids to identify the genetic factors that link pathogenesis and metabolic abnormalities, as well as the discovery of candidate molecules as therapeutic targets.

More information: Masaki Tsuchiya et al, Organelle-selective click labeling coupled with flow cytometry allows pooled CRISPR screening of genes involved in phosphatidylcholine metabolism, *Cell Metabolism* (2023). DOI: 10.1016/j.cmet.2023.02.014

Provided by Japan Science and Technology Agency