Fluorescence Imaging of Fatty Acid Beta Oxidation Pathway in Tissue Sample Using Activity-Based Probe

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Metabolic pathways, chemical reactions that consist of multiple enzyme reactions, play pivotal role for maintaining cellular functions and elucidation of the activity of metabolic pathways is indispensable for understanding of disease mechanism and drug discovery. Fluorescence imaging would be a powerful tool for elucidating heterogeneity of metabolic activity in individual cells. However, targets of current fluorescent probes are generally limited in single enzyme reactions. Because metabolic pathways consist of multiple enzyme reactions, it has been challenging to elucidate activity of whole flux of a certain metabolic pathway using a single fluorescent probe. We here report an activity-based probe for fluorescence imaging of fatty acid beta oxidation (FAO) pathway. FAO is an important metabolic pathway that degrades fatty acids to generate acetyl CoA for ATP production. For fluorescence imaging of FAO, we designed a chemical probe possessing fatty acid moiety that is metabolically degraded by FAO and releases a reactive quinone methide (QM) possessing an alkyne moiety. The QM is covalently trapped by intracellular proteins and the introduction of fluorophore into the labeled proteins enables fluorescence detection of FAO.

Hepatocellular carcinoma HepG2 cells were treated with the probe, fixed and conjugated with TAMRA possessing azide group by CuAAC reaction. Confocal microscopy analysis observed bright fluorescence inside the cells. In contrast, negligible fluorescence was observed in the cells pre-treated with etomoxir, an inhibitor of FAO. We next applied our probe to fluorescence imaging of FAO in mouse liver tissue. Mouse were intraperitoneally administrated with our probe and the liver of the mouse was isolated. The liver slice was treated with TAMRA-azide and subjected to confocal microscopy. Bright fluorescence of TAMRA was observed in the liver slice. In contrast, the fluorescence was effectively suppressed in the liver slice of the mousse pre-treated with etomoxir. These data indicated that our probe enabled detection of FAO not only in cultured cells but also mouse liver tissue.