Development of a Targeted Lipidomics Workflow to Assess Heterogeneous Cellular Responses to Ferroptosis

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Ferroptosis is an iron-dependent, non-apoptotic form of regulated cell death characterized by excessive lipid peroxidation overwhelming endogenous cellular antioxidant defense mechanisms. It is influenced by multiple interconnected cellular pathways, including redox homeostasis, iron metabolism, mitochondrial function, and lipid metabolism. Ferroptotic cell death is ultimately driven by lipid peroxidation of polyunsaturated fatty acid–containing lipids. Cells counteract ferroptosis through several antioxidant defense systems, including glutathione peroxidase 4 and ferroptosis suppressor protein 1. While ferroptosis induction has emerged as a promising therapeutic strategy for treatment-resistant cancers, growing evidence suggests that ferroptosis sensitivity varies substantially across tumor cell populations and between tumor types.[1] Understanding these differences requires analytical approaches capable of resolving lipid changes within specific cellular subpopulations and, ultimately, at the single-cell level. Although single-cell lipidomics approaches are beginning to emerge, these methods remain technically challenging due to the limited abundance of lipids in individual mammalian cells and the sensitivity constraints of conventional liquid chromatography–mass spectrometry (LC-MS) platforms.[2] To address this gap, we will develop and evaluate a targeted lipidomics workflow using a high-sensitivity Waters triple quadrupole LC-MS/MS platform. Initial studies will determine instrument sensitivity limits, lipid coverage, and the minimum number of cells required for reliable targeted lipidomic analysis. The feasibility of single cell lipidomic measurements will then be assessed. If sufficient sensitivity is achieved, single cell lipidomics will be performed directly. Alternatively, fluorescence-activated cell sorting in combination with the lipid peroxidation probe C11-BODIPY and other phenotypic markers will be used to isolate cellular subpopulations exhibiting distinct ferroptotic responses for downstream lipidomic characterization. Establishing this workflow will provide a foundation for investigating ferroptosis-associated cellular heterogeneity and may enable future studies of ferroptosis response and resistance at increasingly refined cellular resolution.

References

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