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ESI-Orbitrap-IRMS Analysis of Free Metabolites for Early Breast Cancer Detection and Insight into Metabolic Mechanisms

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Cancer cells rewire their metabolism as they proliferate, altering pathway activities and metabolite quantities. Isotopic analysis of metabolites in blood plasma could thus work as a non-invasive detection method and, in tissue, provide insight into the cancer metabolic network. Studies have shown cancerous cells to be ^{15}N-depleted and ^{13}C-enriched relative to adjacent non-cancerous cells[1]; however, primary isotopic anomalies occur at atomic sites undergoing bond-breaking and are not fully expressed in molecular average or bulk isotope analysis. With the advent of Orbitrap-IRMS, site-specific isotope analysis (SSIA) is now possible, which targets the atomic sites in the individual metabolites that experience the isotopic effect from the metabolically dysregulated reaction.

In this study, we focus on two metabolic hallmarks of breast cancer metabolism: the Warburg effect and glutamine addiction. We aim to determine whether SSIA of ^{13}C in free metabolites of Lactate and Alanine, and ^{15}N in free metabolites of Glutamine, and Arginine can serve as reliable biomarkers for breast cancer and whether specific isotopic patterns are unique for certain subtypes of breast cancer. Our approach involves three distinct stages: sample purification using reverse-phase HPLC and IC fraction collection, ESI-orbitrap-IRMS measurements, and data processing following the mathematical framework for isotomics. Preliminary quantification from the HPLC of these metabolites in the tissue samples reveals significant differences in cancerous versus adjacent healthy tissue, with cancerous tissues showing multi-fold higher concentrations, along with inter-cancer subtype variations. Using ESI-Orbitrap-IRMS, we achieve sub-permille accuracy on the molecular average for glutamine and approximately 1 permille accuracy for lactate on pure standards, verified by EA measurements. Future work will focus on sample purification and verifying the accuracy of SSIA before measuring biological samples. This study continues the work of SSIA on small quantities of polar organic molecules present in complex matrices. Reliable measurements of the intra-molecular isotopic fingerprints of these metabolites can provide valuable insight into the metabolic mechanisms that sustain cancer cell survival and can be used as a novel breast cancer diagnostic tool.

[1] Tea, I. *et al.* 13C and 15N natural isotope abundance reflects breast cancer cell metabolism. *Sci. Rep.* **6**, 34251 (2016)