

## EDITORIAL

# “METABOLOMICS” IN BREAST CANCER DIAGNOSIS

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Breast cancer, the most frequent cancer in females is the major cause of morbidity and mortality in the world as well as in Pakistan. Screening of breast cancer or early detection requires a reliable biomarker that has high specificity and sensitivity for use in clinical practice. Certain diagnostic biomarker have been identified but are not fit for clinical use due to their low sensitivity like MUC1 (mucin glycoprotein) and CA 15-3 and CA 27-29 (cancer antigens). <sup>1</sup> Several recent review papers have highlighted presence of potential biomarkers identified in blood, ductal lavage, nipple aspirate fluid (NAF), pleural effusion, tissues, fine needle aspiration or core needle biopsy. <sup>2,3</sup>

Early detection and effective diagnosis of breast cancer requires a panel of multiple markers because of the heterogeneous nature of breast cancer. This search for cancer fingerprints has become possible through the use of high-throughput techniques like metabolomics, but also analysis of complex data and pattern recognition through bioinformatics. Potential molecular biomarkers identified by high-throughput methods in current clinical research include DNA sequence variation, micro RNA's or aberrant transcripts, abnormal methylated proteins, metabolites of lipid, carbohydrate or protein metabolism. <sup>4</sup>

Metabolomic analysis include “metabolic profiling” and “metabolic fingerprinting”. Metabolic profiling involves quantitative analysis of metabolites in a specific pathway and the identified metabolites can be assembled as building blocks. Metabolic fingerprinting involves identification of metabolites that shows a change in disease and hence can be used for diagnostic purposes by differential pattern of metabolite expression in normal and diseased individuals. In order to understand the underlying pathogenesis of disease, a powerful metabolomic approach involves performing both metabolic profiling (a quantitative approach) and metabolic fingerprinting (a qualitative approach). This can be a useful and productive approach for identification of a new biomarker. <sup>5</sup>

Analytical techniques used for metabolomics include nuclear magnetic resonance (NMR) spectroscopy, or mass spectrometry (MS). MS can be coupled with different separation techniques e.g. gas chromatography (GC-MS), liquid chromatography (LC-MS), or capillary electrophoresis (CE-MS). GC-MS and LC-MS are the most commonly used techniques in cancer metabolomics. <sup>5</sup> Different mass analyzers can be used for MS like time-of-flight – TOF (Q-TOF, TOF-TOF) and Orbitrap (LTQ-orbitrap) mass analyzers. <sup>6</sup>

Metabolomics in cancer research studies different aspects of cancer metabolism. Metabolic pathways affected in tumor cells include glycolytic pathway, pentose phosphate pathway (PPP), tricarboxylic acid (TCA) cycle, nucleotide and protein biosynthetic pathway, lipid and phospholipid turnover and redox stress pathways. Carbohydrate metabolism has been extensively studied in this regard especially glycolytic pathway, TCA cycle and oxidative phosphorylation. <sup>6</sup>

Tumor cells have an increase in glycolytic activity with increased lactic acid production even in aerobic conditions with a reduction of oxidative phosphorylation and down regulation of TCA cycle. <sup>7</sup> This is called Warburg effect, which hypothesizes that tumor cells suffer hypoxic injury due to reduced oxidative phosphorylation with reduction of ATP. The energy deficit is supplied to tumor cell through fermentation. It also shows an increased uptake of glucose by cancer cells. <sup>8</sup>

A GC-TOF-MS based metabolomic study carried out by Budczies et al reported decreased levels of free fatty acids (FFA), sugars (glucose, fructose, sucrose), benzoic acid family, increased levels of amino acids (esp. glutamine), alterations in TCA cycle and glycerophospholipid metabolism in breast cancer tissue. There was an increase in nucleotide, nucleosides and tumor phosphates in cancer tissues. Furthermore a panel of markers was identified which differentiated between normal breast tissue and breast cancer tissue with a sensitivity and specificity of > 80%. The ratio of cytidine-5-monophosphate to pentadeconoic acid was highly significant amongst all the classifiers with a 94.8% sensitivity and 93.9% specificity. <sup>9</sup>

Similarly Slupsky et al explored the role of urinary metabolomics in non-invasive screening of early breast cancer by nuclear magnetic resonance (NMR) spectroscopy. TCA cycle intermediates, metabolites related to energy metabolism,

amino acid and gut microbial metabolism were specifically altered in urine of breast cancer patients. <sup>10</sup> Another study identified differences between early and metastatic breast cancer patients through differences in their serum metabolomic profile by NMR MS. <sup>11</sup>

Early breast cancer detection is the key to saving precious lives. A single biomarker has low specificity and sensitivity compared to a panel of specific and selective biomarkers which after cross validation can result in the discovery of novel or specific metabolomic signature. With the help of novel high-throughput technology like metabolomics, panels of specific potential biomarker metabolites not only for diagnosis but also for therapy, prognosis and recurrence of breast cancer can be identified. However these new novel biomarkers must be validated before being introduced into clinical practice.

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