Invited critical review

Metabolomics in noninvasive breast cancer

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ABSTRACT

Breast cancer remains the most leading cause of death among women worldwide. Common methods for diagnosis and surveillance include mammography, histopathology and blood tests. The major drawback of mammography is the high rate of false reports, aside from the risk from repeated exposure to harmful ionizing radiations; histopathology is time consuming and often prone to subjective interpretations; blood-based tests are attractive, but lack the sensitivity and specificity. Obviously, more sensitive biomarkers for early detection and molecular targets for better treating breast cancer are urgently needed. Fortunately, molecular level 'omics' diagnosis is becoming increasingly popular; metabolomics, diagnosis based on 'metabolic fingerprinting' may provide clinically useful biomarkers applied toward identifying metabolic alterations and has introduced new insights into the pathology of breast cancer. By applying advanced analytical and statistical tools, metabolomics involves the comprehensive profiling of the full complement of low molecular weight compounds in a biological system and could classify the basis of tumor biology of breast cancer, to identify new prognostic and predictive markers and discover new targets for future therapeutic interventions. This advanced bioanalytic methods may now open new avenues for diagnostics in cancer via discovery of biomarkers. In this review we take a closer look at the metabolomics used within the field of breast cancer diagnosis. Further, we highlight the most interesting metabolomics publications and discuss these in detail; additional studies are mentioned as a reference for the interested reader. A general trend is an increased focus on biological interpretation rather than merely the ability to classify samples.

Contents

1. Introduction

Breast cancer belongs to the most frequent and severe cancer types in women worldwide and its recurrence rates are very high [1]. The burden of breast cancer is growing worldwide and with it a more desperate need for better tools to detect, diagnose and monitor the disease is required. The gold standard method for identifying patients with breast cancer is the mammography, histopathology and blood tests [2–4]. However, these tests are not widely used as a risk assessment tool

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because they are inconvenient, time-consuming, costly and have a poor specificity. Metabolomics, a dynamic portrait of the metabolic status of living systems, offers potential advantages that classically diagnose approaches that did not based on the discovery of a suite clinically relevant biomarker [5,6]. Because small changes in living systems can lead to large changes in metabolite levels, the metabolome can be regarded as the amplified output of a biological system. Monitoring fluctuations of certain metabolites in body fluids, has become an important way to detect early stages in breast cancer [7]. Moreover, metabolomics approaches are likely to be used to screen for potential diagnostic and prognostic biomarkers of breast cancer [8].

Recent metabolomics studies have improved the understanding of the basic mechanisms underlying cancer pathogenesis, which will help to improve treatment strategies [9]. It aimed at characterizing the metabolism of breast cancer to identify new biomarkers and new targets for therapeutic interventions. A comprehensive coverage of metabolism can be achieved by a combination of analytical approaches. The most popular approaches for metabolomics involve mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy [10]. Over the past 10 years, continuous progress in the application of NMR spectroscopy and MS to the detection, diagnosis and characterization of human breast cancer has turned what began as scientific curiosity into a useful clinical option. This technology permits simultaneous monitoring of many hundreds, or thousands, as well as functional monitoring of multiple pivotal cellular pathways. Development of metabolomics platform has made it possible to acquire high-throughput profiles of potential biomarkers [11,12].

Emerging metabolomics is increasingly being used for breast cancer research and personalized medicine; it has provided new opportunities in the molecular analysis of human breast cancer with unprecedented speed and detail [13]. This approach has the potential to provide more information about the pathophysiological status of an organism and distinguish breast cancer stages [14,15]. Metabolomics biomarkers can potentially lead to breast cancer screening and diagnosis and may provide useful information on the cancer type and the disease's stage of progression. It has just begun to enter the mainstream of cancer diagnostics and therapeutics. In this review we intend to explore the potential role of metabolomics in understanding breast cancer process, refining its characterization and searching for predictive biomarkers, highlighting the potential value of metabolomics for the noninvasive analysis of breast cancer.

2. Metabolomics technologies

With technological advances in analytical techniques, the ability to measure low-molecular-weight metabolites in a biofluid provides a powerful platform for identifying metabolites that are uniquely correlated with a specific human disease [16]. Technological developments are the driving force for advances in metabolomics, and identifying novel changes in specific metabolites. A key task in cancer medicine is to detect the disease as early as possible. In order to achieve this, many new technologies have been developed for cancer biomarker discovery [17]. Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the main analytical spectroscopic approaches in metabolic profiling, usually offering complementary information, but with different performance characteristics. Their complementary nature makes the combination particularly attractive and can provide complementary snapshots of the metabolome of body fluids such as plasma, urine or cerebrospinal fluid [18]. High-definition MS has been carried out to obtain comprehensive metabolite profiling and pathways of large biological data sets. MS tends to have much higher analytical sensitivity, and enables broader surveys of the metabolome either in a targeted or nontargeted manner. Because no single analytical method can accommodate the chemical diversity of the entire metabolome; thus, a multiplatform approach may provide a more comprehensive

understanding of metabolic alterations [19]. A combined analytical approach can improve the potential for providing reliable methods to detect metabolic profile alterations in a biological specimen. In the study by Asiago et al., using a combination of NMR and GC × GC–MS methods, the metabolite profiles of 257 serum samples from 56 breast cancer patients were analyzed and eleven metabolite markers provided a sensitivity of 86% and a specificity of 84% [20]. The combination of two advanced analytical methods, NMR and MS, provides a powerful approach for the early detection of recurrent breast cancer. Additionally, an improved metabolic profile obtained by combining MS and NMR approach may be useful to achieve more accurate disease detection and gain more insight regarding breast cancer mechanisms and biology [21].

3. Potential role of metabolites

Metabolomics capitalizes on the unique presence and concentration of small molecules in body fluids to construct a 'fingerprint' that can be unique to the individuals, including health and disease states. It has been increasingly applied to discover biomarkers, identify perturbed pathways, diagnose diseases, and measure the response to treatment [22]. In the area of breast cancer, the integrated analysis of metabolites may provide a powerful platform for detecting changes related to cancer diagnosis and discovering novel biomarkers [23]. Biomarkers are biological characteristics that are objectively measured and evaluated as indicators of pathological processes [24]. It is a biological molecule found in body fluids that can be a sign of a disease, and has been widely used in clinical practice for the diagnosis, assessment of severity and response to therapy in a number of clinical disease states.

Analyzing metabolic differences between unperturbed and perturbed systems, such as healthy volunteers and patients with a disease, can lead to insights into the underlying pathology [25]. Assessment of a biological system by means of global and non-targeted metabolomics provides the investigator with molecular information that is close to the phenotype. Biomarker discovery is one of the newly emerging innovations in the diagnosis and treatment of cancer and many other diseases. With advances in methods and technology, together with the considerable efforts to find early and novel diagnostic breast cancer biomarkers, many candidates will be discovered; leading to early diagnosis, detection, monitoring and efficient treatment of breast cancers [26]. More specifically, metabolomics has a global and non-invasive analysis of biomarkers that are indicators of pathogenic process, thereby helping to monitor treatment response [27]. Recently, a variety of multiple biomarkers reflecting breast cancer pathologies have been developed and have the potential to serve an important role in diagnosis and management of human conditions.

4. Metabolomics features of breast cancer

Understanding the metabolome will not only provide insights into the critical sites of regulation in health promotion, but will also assist in identifying intermediate or surrogate cancer biomarkers for establishing preventative or therapeutic approaches for health. Metabolomics increased our knowledge of the dysregulated metabolic pathways associated with progression of diseases and provided potentially new therapeutic strategies targeting these pathways, through our understanding about the distinct and complete metabolic footprints of breast cancer [28]. Over the last few years, there has been a rapidly growing number of metabolomics applications aimed at finding biomarkers which could assist diagnosis, provide therapy guidance, and evaluate response to therapy for particular breast cancer. Metabolite changes that were observed in diseased individuals as a primary indicator have been an important part of clinical practice [29]. This dynamic, simultaneous assessment of thousands of metabolites allows identification of the presence, concentration and fluxes of specific metabolites, and recognition of the critical metabolic pathways recruited in carcinogenesis.

Elucidation of these metabolites and pathways may provide essential insights into both the intercellular environment and host/tumor interaction, allowing recognition of new biomarkers for diagnosis and prediction of outcome, and new therapy targets. Metabolomics has recently moved into one of the cornerstones of postgenomics for the quantitative analysis and unbiased identification of small molecular metabolites which could yield important information about a person's disease [30].

5. Bringing metabolomics into breast cancer research

Some studies have demonstrated that patient outcome of breast cancer is substantially influenced by cancer stage at the time of diagnosis [31]. For example, patients with early stage breast cancer have a significant higher 5-year survival rates compared to patients diagnosed at late stage. Thus, it is important to develop effective methods for early diagnosis as well as for precise staging of this disease process. Although traditional test remains the most effective means to diagnose breast cancer, this approach generally suffers from poor patient compliance. As such, it is imperative to develop accurate and specific tests that utilize more convenient approaches. Many diseases result in specific and characteristic changes in the biochemical profiles of biological fluids prior to the development of clinical symptoms [32]. These changes are often useful diagnostic and prognostic biomarkers. Identifying biomarkers that can be used for the early detection of breast cancer will result in more efficient treatments, reduction in suffering, and lower mortality rates. An ideal screening test should be non-invasive with high sensitivity and specificity. Metabolomics is the upcoming new science in the omics field with the potential to further increment our knowledge of cancer biology [33]. It has recently emerged as a novel method of breast cancer detection owing to its ability to monitor changes in the metabolic signature that reflect changes in phenotype and function [34].

The translational value of metabolomics in the breast cancer studies has been demonstrated by the identification of diagnostic and prognostic biomarkers [35–38]. To become a clinically approved test, a potential biomarker should be confirmed and validated using hundreds of specimens and should be reproducible, specific, and sensitive. The accumulation of information from novel metabolomics technologies comes with substantial hope and expectations that these approaches will yield novel insights into breast cancer processes [39,40]. Integration of metabolomics-based diagnostic principles into the breast cancer might be the direction to enable a revolution for future health care, also perhaps it is time to embrace the arrival of 'Breast Cancer-OMICS' era.

6. Metabolomics studies on breast cancer

Metabolomics has potential power to enhance our understanding of the root causes of breast cancer, and provides a detailed snapshot of the body's processes at any particular point in time, and opens up the possibility of monitoring health and disease, prevention and treatment. In order to expand the metabolite profiling capabilities to breast cancer, several recent studies have developed. The metabolites as the end products of cellular processes, are closely linked to phenotypes. Five potential urinary biomarkers were successfully identified for breast cancer with high accuracy [41]. The combinatorial effects among multiple biomarkers can enhance discriminative power for breast cancer. The proposed urine metabolomics method was applied to select candidate biomarkers from 50 breast cancer patients and 50 normal persons [42]. Among the altered metabolic pathways, four metabolic biomarkers such as homovanillate, 4-hydroxyphenylacetate, 5-hydroxyindoleacetate and urea, were identified. Urine samples from 85 breast cancer women and healthy controls were analyzed to assess the metabolic profiles of nucleosides [43], and a valid set of 35 candidates was selected. Based on this approach, ultimate

estimates for sensitivity and specificity of 83.5% and 90.6% were obtained for best prediction of breast cancer.

A comprehensive metabolic map of breast cancer was constructed by GC–TOFMS analysis [44]. A total number of 368 were detected and significantly changed between cancer and normal tissues. These metabolites are promising biomarkers for medical screening. Specifically, the cytidine-5-monophosphate/pentadecanoic acid ratio was the most significant discriminator and allowed detection with a sensitivity of 94.8% and a specificity of 93.9%. Changes in lipid metabolism are an important and characterized hallmark of cancer. Metabolomics analysis by GC–MS showed that sn-glycerol-3-phosphate, was elevated in breast cancer compared to normal breast tissue [45]. In a subsequent study, Li M et al. performed metabolomics studies on breast tissue samples [46]. It showed that cancer and non-cancer samples can be discriminated very well with OPLS-DA multivariate model. A subsequent blind test showed 69% sensitivity and 94% specificity in the prediction of the cancer status. A spectral analysis showed that in taurine- and choline-containing compounds are elevated. In the study done by Giskeødegård GF et al., the relationship between the metabolite profiles of breast cancer tissue and 5-year survival had been examined [47]. Higher levels of glycine and lactate were found to be associated with lower survival rates by both multivariate analyses and spectral integration, and are suggested as biomarkers for breast cancer prognosis. Results imply that the metabolic state of a tumor may provide additional information concerning breast cancer prognosis.

Metabolic phenotypes of breast cancers in urine were investigated [48]. Intermediates of the tricarboxylic acid cycle and metabolites relating to energy metabolism, amino acids, and gut microbial metabolism were perturbed and illustrated that urinary metabolomics may be useful for detecting early-stage breast cancer. Forty-four early breast cancer patients with pre- and postoperative serum samples had metabolomics assessment [49]. Innate serum metabolomics differences exist between early and metastatic patients. Preoperative patients were identified with 75% sensitivity, 69% specificity and 72% predictive accuracy. Biomarkers are emerging as determinants of breast cancer prognosis. Work done by Simpson et al. demonstrated that sensitive histone modifications and corresponding histone modifying enzymes can be used as diagnostic and prognostic biomarkers for breast cancer [50].

The non-targeted qualitative profiling was first achieved to get metabolic patterns of collected samples and the targeted quantitative analysis focused on hormonal metabolism was also conducted [51]. Two known biomarkers, i.e., 5-hydroxymethyl-2-deoxyuridine and 8-hydroxy-2-deoxyguanosine, in breast cancer were also confirmed using the present methods. These cancer markers are highly related to metabolites which are responsible for oxidative DNA damage and DNA methylation process. A high resolution MS-based metabolomics has been implemented to find biologically significant metabolite biomarkers in breast cancer [52]. Finally, a set of 12 metabolites was identified as potential biomarkers including amino acids, organic acids, and nucleosides, and revealed elevated tryptophan and nucleoside metabolism as well as protein degradation in breast cancer patients. It demonstrates the advantages of integrating metabolic networks with metabolomics for finding significant potential biomarkers.

Metabolomics, a high-throughput global metabolite analysis, has shown substantial evidence to support its emerging role in breast cancer diagnosis, recurrence, prognosis, and identifying novel cancer biomarkers as well as developing cancer therapeutics [53]. Recent developments in the area of metabolic analysis may help to close the gap between clinical metabolomics research and the development of cancer metabolome [54]. Any findings associated with relevance to breast cancer, once passed to the clinical level, will be eventually combined with other diagnostic approaches to hopefully reach the 100% detection level for high-risk patients. Recent advances in metabolomics along with the novel strategies to analyze, understand,

and construct the metabolic pathways open this window of opportunity in a very effective manner. We predict an intensified use of metabolomics screens in clinical and preclinical studies focusing on the onset and progression of breast cancer development.

7. Conclusions and future perspectives

Elimination of cancer in the 21st century is likely to depend not only on more effective individualized treatment, but also upon earlier detection and prevention. Breast cancer is the most common cancer in women worldwide and the high prevalence of breast cancer has caused a huge burden on the modern society, and the development of new technologies for better understanding of the molecular changes is essential. There is still a lack of reliable biomarkers indicative of metabolic alterations, highlighting the need for the development of early diagnostic and prognostic markers for breast cancer. Metabolomics has the potential to generate novel noninvasive diagnostic tests, based on biomarkers of disease, which are simple and cost effective yet retain high sensitivity and specificity characteristics. In future work, these potential biomarkers should be further validated with a large enough patient cohort to achieve earlier diagnosis of breast cancer. To enable a better stratification of patients, it is important to identify the pathways that are relevant for tumor progression and therapy response and to determine biomarkers that could be used to monitor the activity of those pathways. A deeper understanding of global perturbations in biochemical pathways could provide valuable insights about mechanisms of disease, prognostic, and diagnostic biomarkers. It will lead to new insights into the mechanisms of cancer progression and cancer patient management through improved molecular diagnostics leading to improved therapeutic concepts by selection of effective drugs as part of systems medicine. This improves the likelihood that genuine metabolic biomarkers for breast cancer can be detected and validated, and will eventually lead to diagnostic toolkits that will facilitate a much more precise predictive and prognostic assessment.

Competing interests

The authors have declared that they have no competing interests.

References

[1] Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, et al. Genes that mediate breast cancer metastasis to the brain. Nature 2009;459(7249):1005–9.

[2] Shi L. Racial differences in breast cancer patterns. Science 2010;329:5987.

[3] Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, et al. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. Nature 2011;476(7360):346–50.

[4] Genetics Couzin J. DNA test for breast cancer risk draws criticism. Science 2008;322(5900):357.

[5] Locasale JW, Grassian AR, Melman T, Lyssiotis CA, Mattaini KR, Bass AJ, et al. Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. Nat Genet 2011;43(9):869–74.

[6] Perez EA. Breast cancer management: opportunities and barriers to an individualized approach. Oncologist 2011;16(Suppl. 1):20–2.

[7] Tenori L, Oakman C, Claudino WM, Bernini P, Cappadona S, Nepi S, et al. Exploration of serum metabolomic profiles and outcomes in women with metastatic breast cancer: a pilot study. Mol Oncol 2012;6(4):437–44.

[8] Klawitter J, Klawitter J, Gurshtein J, Corby K, Fong S, Tagliaferri M, et al. Bezielle (BZL101)-induced oxidative stress damage followed by redistribution of metabolic fluxes in breast cancer cells: a combined proteomic and metabolomic study. Int J Cancer 2011;129(12):2945–57.

[9] Zhang A, Sun H, Wu X, Wang X. Urine metabolomics. Clin Chim Acta 2012;414:65–9.

[10] Arakaki AK, Skolnick J, McDonald JF. Marker metabolites can be therapeutic targets as well. Nature 2008;456:443.

[11] Zhang A, Sun H, Yan G, Han Y, Ye Y, Wang X. Urinary metabolic profiling identifies a key role for glycocholic acid in human liver cancer by ultra-performance liquid-chromatography coupled with high-definition mass spectrometry. Clin Chim Acta 2013;418:86–90.

[12] Zhang A, Sun H, Han Y, Yuan Y, Wang P, Song G, et al. Exploratory urinary metabolic biomarkers and pathways using UPLC–Q-TOF–HDMS coupled with pattern recognition approach. Analyst 2012;137(18):4200–8.

[13] Yuan M, Breitkopf SB, Yang X, Asara JM. A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. Nat Protoc 2012;7:872–81.

[14] Nicholson JK, Wilson ID. Understanding 'global' systems biology: metabonomics and the continuum of metabolism. Nat Rev 2003;2:668–76.

[15] Chen F, Xue J, Zhou L, Wu S, Chen Z. Identification of serum biomarkers of hepatocarcinoma through liquid chromatography/mass spectrometry-based metabonomic method. Anal Bioanal Chem 2011;401(6):1899–904.

[16] Sun H, Zhang A, Yan G, Piao C, Li W, Sun C, et al. Metabolomic analysis of key regulatory metabolites in hepatitis C virus-infected tree shrews. Mol Cell Proteomics 2013;12(3):710–9.

[17] Patterson AD, Maurhofer O, Beyoglu D, Lanz C, Krausz KW, Pabst T, et al. Aberrant lipid metabolism in hepatocellular carcinoma revealed by plasma metabolomics and lipid profiling. Cancer Res 2011;71(21):6590–600.

[18] Zhang A, Sun H, Wang X. Saliva metabolomics opens door to biomarker discovery, disease diagnosis, and treatment. Appl Biochem Biotechnol 2012;168(6):1718–27.

[19] Wang X, Zhang A, Sun H. Power of metabolomics in diagnosis and biomarker discovery of hepatocellular carcinoma. Hepatology 2013;57(5):2072–7.

[20] Asiago VM, Alvarado LZ, Shanaiah N, Gowda GA, Owusu-Sarfo K, Ballas RA, et al. Early detection of recurrent breast cancer using metabolite profiling. Cancer Res 2010;70(21):8309–18.

[21] Fukushima A, Kusano M, Nakamichi N, Kobayashi M, Hayashi N, Sakakibara H, et al. Impact of clock-associated Arabidopsis pseudo-response regulators in metabolic coordination. Proc Natl Acad Sci U S A 2009;106:7251–6.

[22] Catchpole GS, Beckmann M, Enot DP, Mondhe M, Zywicki B, Taylor J, et al. Hierarchical metabolomics demonstrates substantial compositional similarity between genetically modified and conventional potato crops. Proc Natl Acad Sci U S A 2009;102:14458–62.

[23] Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature 2009;457:910–4.

[24] Wang X, Zhang A, Han Y, Wang P, Sun H, Song G, et al. Urine metabolomics analysis for biomarker discovery and detection of jaundice syndrome in patients with liver disease. Mol Cell Proteomics 2012;11(8):370–80.

[25] Gu H, Pan Z, Xi B, Asiago V, Musselman B, Raftery D. Principal component directed partial least squares analysis for combining nuclear magnetic resonance and mass spectrometry data in metabolomics: application to the detection of breast cancer. Anal Chim Acta 2011;686(1–2):57–63.

[26] Malet-Martino M, Holzgrabe U. NMR techniques in biomedical and pharmaceutical analysis. J Pharm Biomed Anal 2011;55(1):1–15.

[27] Weljie AM, Bondareva A, Zang P, Jirik FR. (1)H NMR metabolomics identification of markers of hypoxia-induced metabolic shifts in a breast cancer model system. J Biomol NMR 2011;49(3-4):185–93.

[28] Oakman C, Tenori L, Biganzoli L, Santarpia L, Cappadona S, Luchinat C, et al. Uncovering the metabolomic fingerprint of breast cancer. Int J Biochem Cell Biol 2011;43(7):1010–20.

[29] Vermeer LS, Fruhwirth GO, Pandya P, Ng T, Mason AJ. NMR metabolomics of MTLn3E breast cancer cells identifies a role for CXCR4 in lipid and choline regulation. J Proteome Res 2012;11(5):2996–3003.

[30] Zhang A, Sun H, Wang X. Serum metabolomics as a novel diagnostic approach for disease: a systematic review. Anal Bioanal Chem 2012;404(4):1239–45.

[31] Bailey ST, Shin H, Westerling T, Liu XS, Brown M. Estrogen receptor prevents p53-dependent apoptosis in breast cancer. Proc Natl Acad Sci U S A 2012;109(44):18060–5.

[32] Klawitter J, Shokati T, Moll V, Christians U, Klawitter J. Effects of lovastatin on breast cancer cells: a proteo-metabonomic study. Breast Cancer Res 2010;12(2):R16.

[33] Sitter B, Bathen TF, Singstad TE, Fjøsne HE, Lundgren S, Halgunset J, et al. Quantification of metabolites in breast cancer patients with different clinical prognosis using HR MAS MR spectroscopy. NMR Biomed 2010;23(4):424–31.

[34] Kleiner M, Wentrup C, Lott C, Teeling H, Wetzel S, Young J, et al. Metaproteomics of a gutless marine worm and its symbiotic microbial community reveal unusual pathways for carbon and energy use. Proc Natl Acad Sci U S A 2012;109(19):E1173–82.

[35] Rivas-Ubach A, Sardans J, Pérez-Trujillo M, Estiarte M, Peñuelas J. Strong relationship between elemental stoichiometry and metabolome in plants. Proc Natl Acad Sci U S A 2012;109(11):4181–6.

[36] Wu R, Wu Z, Wang X, Yang P, Yu D, Zhao C, et al. Metabolomic analysis reveals that carnitines are key regulatory metabolites in phase transition of the locusts. Proc Natl Acad Sci U S A 2012;109(9):3259–63.

[37] Finley LW, Lee J, Souza A, Desquiret-Dumas V, Bullock K, Rowe GC, et al. Skeletal muscle transcriptional coactivator PGC-1α mediates mitochondrial, but not metabolic, changes during calorie restriction. Proc Natl Acad Sci U S A 2012;109(8):2931–6.

[38] Mintz-Oron S, Meir S, Malitsky S, Ruppin E, Aharoni A, Shlomi T. Reconstruction of Arabidopsis metabolic network models accounting for subcellular compartmentalization and tissue-specificity. Proc Natl Acad Sci U S A 2012;109(1):339–44.

[39] Lange BM, Mahmoud SS, Wildung MR, Turner GW, Davis EM, Lange I, et al. Improving peppermint essential oil yield and composition by metabolic engineering. Proc Natl Acad Sci U S A 2011;108(41):16944–9.

[40] Ward JL, Baker JM, Llewellyn AM, Hawkins ND, Beale MH. Metabolomic analysis of *Arabidopsis* reveals hemiterpenoid glycosides as products of a nitrate ion-regulated, carbon flux overflow. Proc Natl Acad Sci U S A 2011;108(26):10762–7.

[41] Kim Y, Koo I, Jung BH, Chung BC, Lee D. Multivariate classification of urine metabolome profiles for breast cancer diagnosis. BMC Bioinformatics 2010;11(Suppl.2):S4.

[42] Nam H, Chung BC, Kim Y, Lee K, Lee D. Combining tissue transcriptomics and urine metabolomics for breast cancer biomarker identification. Bioinformatics 2009;25(23):3151–7.

[43] Henneges C, Bullinger D, Fux R, Friese N, Seeger H, Neubauer H, et al. Prediction of breast cancer by profiling of urinary RNA metabolites using support vector machine-based feature selection. BMC Cancer 2009;9:104.

[44] Budczies J, Denkert C, Müller BM, Brockmöller SF, Klauschen F, Györffy B, et al. Remodeling of central metabolism in invasive breast cancer compared to normal breast tissue — a GC–TOFMS based metabolomics study. BMC Genomics 2012;13:334.

[45] Brockmöller SF, Bucher E, Müller BM, Budczies J, Hilvo M, Griffin JL, et al. Integration of metabolomics and expression of glycerol-3-phosphate acyltransferase (GPAM) in breast cancer—link to patient survival, hormone receptor status, and metabolic profiling. J Proteome Res 2012;11(2):850–60.

[46] Li M, Song Y, Cho N, Chang JM, Koo HR, Yi A, et al. An HR-MAS MR metabolomics study on breast tissues obtained with core needle biopsy. PLoS One 2011;6(10):e25563.

[47] Giskeødegård GF, Lundgren S, Sitter B, Fjøsne HE, Postma G, Buydens LM, et al. Lactate and glycine-potential MR biomarkers of prognosis in estrogen receptor-positive breast cancers. NMR Biomed 2012;25(11):1271–9.

[48] Slupsky CM, Steed H, Wells TH, Dabbs K, Schepansky A, Capstick V, et al. Urine metabolite analysis offers potential early diagnosis of ovarian and breast cancers. Clin Cancer Res 2010;16(23):5835–41.

[49] Oakman C, Tenori L, Claudino WM, Cappadona S, Nepi S, Battaglia A, et al. Identification of a serum-detectable metabolomic fingerprint potentially correlated with the presence of micrometastatic disease in early breast cancer patients at varying risks of disease relapse by traditional prognostic methods. Ann Oncol 2011;22(6):1295–301.

[50] Simpson NE, Tryndyak VP, Beland FA, Pogribny IP. An in vitro investigation of metabolically sensitive biomarkers in breast cancer progression. Breast Cancer Res Treat 2012;133(3):959–68.

[51] Woo HM, Kim KM, Choi MH, Jung BH, Lee J, Kong G, et al. Mass spectrometry based metabolomic approaches in urinary biomarker study of women's cancers. Clin Chim Acta 2009;400(1–2):63–9.

[52] Chen Y, Zhang R, Song Y, He J, Sun J, Bai J. RRLC–MS/MS-based metabonomics combined with in-depth analysis of metabolic correlation network: finding potential biomarkers for breast cancer. Analyst 2009;134(10):2003–11.

[53] Nomura DK, Dix MM, Cravatt BF. Activity-based protein profiling for biochemical pathway discovery in cancer. Nat Rev Cancer 2010;10(9):630–8.

[54] Tomita M, Kami K. Cancer. Systems biology, metabolomics, and cancer metabolism. Science 2012;336(6084):990–1.