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Metabolic signatures of human breast cancer

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Abbreviations: ER, estrogen receptor; PC, phosphatidylcholine; LPA, lysophosphatidic acid; 2HG, 2-hydroxyglutarate; ROS, reactive oxygen species; TCA, tricarboxylic acid.

Metabolomics has emerged as a new discovery tool with the promise of identifying therapeutic targets in cancer. Recent discoveries have described essential metabolomic pathways in breast cancer and characterized oncometabolites that drive tumor growth and progression. Oncogenes like *MYC* and tumor suppressor genes like *TP53* prominently affect breast cancer biology through regulation of cell metabolism and mitochondrial biogenesis. These findings indicate that tumors with dominant mutations could be susceptible to inhibitors of disease metabolism. Moreover, various preclinical and clinical studies have linked tumor metabolism to therapeutic response and patient survival. Thus, recent advances suggest that metabolic profiling provides new opportunities to improve outcomes in breast cancer. In this review we summarize some of the identified roles of oncometabolites in breast cancer biology and highlight their clinical utility.

Introduction

Malignant transformation induces reprogramming of cell metabolism to support tumor growth, tissue remodeling, and cancer metastasis. This switch is regulated by oncogenes and tumor suppressor genes and is influenced by the tumor microenvironment. In turn, cancer cell metabolism can alter the function of stromal cells, induce tumor vascularization and inflammation, inhibit the immune response, and reduce the efficacy of cancer therapy. In breast cancer, large differences in tissue metabolite profiles have been observed between estrogen receptor (ER)-positive and ER-negative tumors, however these differences do not appear to further classify tumors into the molecular subtypes that were described from gene expression profiling studies.[1–3] Instead, oncogenic *MYC* and the *TP53* tumor suppressor gene seem to have major effects on metabolism in breast tumors.[2,3] Additionally, tumor glutamine and glutamate levels may describe subsets of tumors that might respond favorably to inhibitors of glutaminolysis.[2,4] Recent research identified several key oncometabolites that are associated with cancer progression. In breast cancer, glutamine (as a substrate) and lactate (as an end product) enhance disease aggressiveness and constitute candidate targets for breast cancer therapy. Other studies suggest lipolytic enzymes as targets for potential intervention because their activity leads to the release of oncogenic lipid messengers such as various lysolipids, lysophosphatidic acid, and eicosanoids that induce breast cancer metastasis. Additional findings revealed that ER-negative breast tumors are commonly dependent on serine synthesis for tumor growth. These and other tumors may also abnormally accumulate various phospholipids or the oncometabolite 2-hydroxygluratate. In this review, we will discuss in detail recent advances in our understanding of breast cancer metabolism and highlight the key pathways of metabolic reprogramming. Furthermore, we will describe how these pathways contribute to tumor biology and can be therapeutically altered to improve outcomes.

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Oncometabolites in Breast Cancer Biology

Normal metabolism is required to maintain tissue homeostasis. Changes in metabolism can either predispose to disease or can be acquired during the process of disease development. Notably, metabolism can greatly vary from person to person. These differences can have genetic causes and contribute to disease risk. They may also affect the course of a disease or lead to an adverse drug response. It has recently been shown that interindividual variations in blood metabolite levels can be linked to common germline genetic variations.[5,6] These variations may therefore constitute inherited risk factors, and they should be further studied in the context of complex diseases like cancer. On the other hand, some metabolites that affect tumor biology may have a microbial origin. Deoxycholate, which is synthesized by bacteria in the intestine, accumulates in human breast tissue and was found to promote survival of breast cancer cells at low micromolar concentrations but induce apoptosis at higher concentrations.[7]

Tumors acquire persistent changes in metabolism during disease development and may become metabolite addicted, which can be exploited in cancer therapy.[8] For example, breast tumors commonly develop a lipogenic phenotype and heavily rely on glucose and glutamine consumption for tumor growth. This reprogramming of cell metabolism in breast cancer is facilitated by oncogenes and tumor suppressor genes and both catalytic and

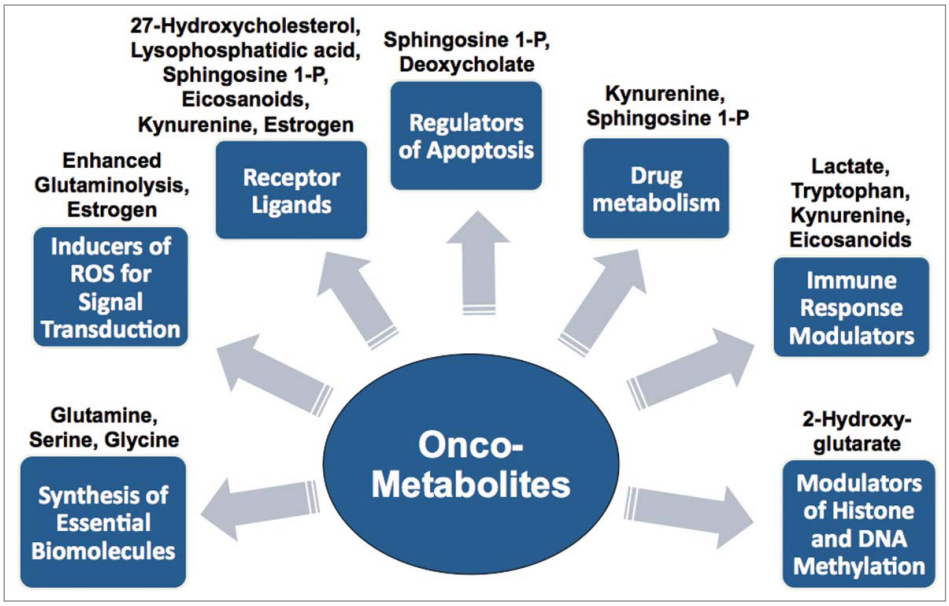

Figure 1. Examples of putative oncogenic metabolites ("oncometabolites") in breast cancer. Increased availability and aberrant accumulation of these metabolites can enhance tumor growth and metastasis. Shown are functional classes of oncometabolites and how they affect breast cancer biology. Most metabolites are generated within breast tumors but some originate from distant organs and reach the breast tissue through the blood supply (e.g., 27-hydroxycholesterol, glutamine, deoxycholate, tryptophan). Deoxycholate has a microbial origin.

noncatalytic roles of enzymes. This process assigns important functions to a subset of metabolites, termed oncometabolites, in cancer biology and disease progression. These oncometabolites can have vital metabolic functions in normal cells but support malignant transformation through various mechanisms (**Fig. 1**). Most of them support cell growth by providing building stones for the synthesis of essential biomolecules, such as glucose and fatty acids. Others have a key role in protecting cancer cells from excessive damage by reactive oxygen species (ROS), such as NADPH, cysteine, glycine, and unsaturated fatty acids. Yet another class of metabolites function as signal transduction molecules. Prostaglandins constitute a class of well-known oncogenic lipid messengers that link cyclooxygenase-2, a key enzyme in prostaglandin synthesis that is aberrantly increased in breast tumors, to an aggressive disease phenotype.[9–11] Recently, 27-hydroxycholesterol (27HC) has been described as a novel estrogen receptor (ER) ligand that promotes cell-autonomous growth of ER-positive tumors.[12,13] 27HC is not aberrantly produced by the tumor itself but links hypercholesterolemia to breast cancer pathophysiology. Lysophosphatidic acid (LPA) is the product of autotaxin, a lysophospholipase that is known as an autocrine motility factor. LPA binds to G-protein-coupled surface receptors that are commonly upregulated in breast cancer.[14] LPA-induced receptor signaling then increases breast tumorigenesis, invasion, and metastasis. This pathway is now being targeted by LPA receptor antagonists in an effort to abrogate the metastatic spread of breast cancer.[15]

Kynurenine is a tryptophan metabolite and signaling molecule that was shown to be an endogenous ligand of the human aryl hydrocarbon receptor (AHR).[16] AHR activation suppresses cellular immune responses and promotes cancer development. It also leads to upregulation of important phase 1 and 2 metabolism enzymes that have key functions in cancer drug metabolism. Kynurenine is synthesized by the indolamine-2,3-dioxygenase, a metabolic enzyme and therapeutic target with increased expression in various cancers including breast cancer.[17] 2-Hydroxyglutarate (2HG) accumulates to high levels in tumors with mutations in isocitrate dehydrogenase 1 and 2 (IDH)[18,19] and was also found to be accumulated 50- to 200-fold in a subset of human breast tumors that do not harbor IDH mutations.[2,3] 2HG affects cancer biology at least in part as a competitive inhibitor of α-ketoglutarate-dependent dioxygenases.[20,21] Accumulation of 2HG leads to aberrations in DNA and histone methylation that cause a reversible dedifferentiation into a stem cell-like phenotype.[19,21] Hence, changes in metabolite patterns in breast tumors that have been described in numerous investigations[1,2,22–26] may affect disease biology in complex ways. Furthermore, these oncometabolites may promote tumorigenesis by changing the differentiation status of tumors and inducing a metastatic phenotype, or by making tumors more viable in stress situations that arise with the excessive release of deleterious oxygen and nitrogen radicals during hypoxia or therapy.

The Basic Pathways

The metabolism of breast tumors, like that of most cancers, heavily relies on the use of both aerobic glycolysis and glutamine catabolism to support cancer cell growth.[27,28] Both pathways are prospective targets in breast cancer therapy.[8,29] Aerobic glycolysis metabolizes glucose to provide cells with acetyl-CoA and NADPH that are required for the synthesis of larger molecules such as lipids, proteins, and nucleic acids. Moreover, acetyl-CoA induces cell growth by directly affecting acetylation of histones at loci that encode growth-related genes, leading to increased transcription of these genes.[30] Aerobic glycolysis bypasses mitochondrial oxidative phosphorylation to avoid an unbalanced and detrimental overproduction of ATP and NADH.[27] Aerobic glycolysis also produces large quantities of lactate to regenerate and maintain an essential NAD+ pool. Lactate is secreted into the tumor microenvironment where it acidifies the extracellular space, increases angiogenesis, and modulates phenotypes of stromal cells in support of continuous tumor growth.[31]

Like glucose, glutamine is taken up by cancer cells and has a crucial role in the replenishment of the mitochondrial citric acid

carbon pool. Glutamine is converted into glutamate, which is a precursor for glutathione. It is also a source for the synthesis of other amino acids and α-ketoglutarate, a tricarboxylic acid (TCA) cycle intermediate and substrate for various dioxygenases including prolyl hydroxylases, histone demethylases, and 5-methylcytosine hydroxylases.[28] Glutaminolysis may directly feed into the TCA cycle but it can alternatively be used to feed into a process termed reductive carboxylation of glutamine-derived α-ketoglutarate that constitutes a partial reverse of the TCA cycle to support citrate and fatty acid synthesis in stress situations such as hypoxia.[32,33] In addition, mitochondrial glutamine may serve as a source for 2HG synthesis in breast tumors.[2] Glutamine restriction slows the growth of most breast cancer cells and targeting the activity of mitochondrial glutaminase, a key enzyme in glutamine metabolism, induces growth arrest and apoptosis, especially among breast cancer cells with the triple-negative phenotype.[29,34–36] However, the metabolic requirements for glutamine can vary substantially among breast cancer cell lines, and some do not depend on glutamine for mitochondrial respiration or survival.[35,36] Instead, inhibition of the cystine/glutamate exchange activity by targeting the xCT antiporter in a subset of glutamine-reliant cells leads to inhibition of cell growth in triple-negative breast cancer cell lines.[36] In such cases, glutamine consumption is used for cystine uptake and glutathione synthesis rather than fueling the TCA cycle and respiration.

A Central Role of Serine Metabolism in Tumor Growth

Glutamine metabolism in breast tumors is linked to several other common metabolic aberrations in breast cancer. A prime example is the serine metabolism pathway. The enzyme phosphoglycerate dehydrogenase (PHGDH) is a novel oncogene that is frequently amplified in ER-negative breast tumors.[37,38] Increased expression of PHGDH facilitates the diversion of glycolytic carbon into serine and glycine metabolism. Alternatively, knockdown of PHGDH inhibits cell growth, indicating that this pathway is a candidate therapeutic target in breast cancer. An increased serine pathway flux through PHGDH supports cell growth partially because it provides a source for glycine synthesis, and glycine by itself has a significant role in enhancing cancer cell proliferation.[39] However, recent data indicate that maintaining a replenished α-ketoglutarate pool in cancer cells is probably a key function of PHGDH since knockdown of this enzyme causes a large drop in α-ketoglutarate levels.[37] Moreover, because the serine synthesis pathway is functionally linked to the anaplerosis of glutamine-derived carbon via the phosphoserine aminotransferase 1 reaction, an increased level of PHGDH has a major impact on glutamine-derived α-ketoglutarate production and becomes a driver of α-ketoglutarate synthesis, mainly in ER-negative breast cancer.[37] Thus, targeting PHGDH with enzyme-specific inhibitors may slow disease progression by reducing the availability of both glycine and glutamine-derived α-ketoglutarate. In addition, serine is a ligand of pyruvate kinase M2 (PKM2) and activates this key glycolytic enzyme whereas serine starvation leads to reduced PKM2 activity and TP53-dependent metabolic remodeling.[40,41] PKM2 is a splice isoform of pyruvate kinase and the predominant isoform in cancer cells. It diverts glucose into aerobic glycolysis but is also a sensor of oxidative stress that contributes to the cellular antioxidant response and thereby protects cells from oxidative damage.[27,42,43] In the MMTV-NeuT mouse breast cancer model, an isoform switch from PKM1 to PKM2 was shown to take place during tumorigenesis whereas inhibition of PKM2 slowed the growth of breast cancer cells. These studies suggest that selective inhibition of PKM2 may have potential as a therapeutic intervention in breast cancer.[42] However, subsequent research discovered that tumor development is not dependent on PKM2 expression in a *Brca1* loss-driven breast cancer model.[44] In this animal model with dual deletion of *Pkm2* and *Brca1* in the tumors, proliferating tumor cells tended to have low levels of PKM1, which in human tumors is achieved by the well-characterized isoform switch in expression from PKM1 to PKM2 and by maintaining PKM2 in an inactive or low activity state. Although data from the mouse model do not endorse inhibition of PKM2 enzyme activity as an anticancer therapy, PKM2 may have undefined oncogenic functions in humans. For example, it was shown to activate HIF1α by protein–protein interactions.[45]

Lipid Metabolism is a Driver of Disease Pathogenesis

Breast tumors, like most cancers, develop a lipogenic phenotype and show an aberrant pattern in the synthesis of fatty acids, membrane phospholipids, and lysophospholipids.[22,24,46–48] Most evident is the fatty acid synthase (FASN)-driven lipogenesis that provides fatty acid precursors for aberrant phospholipid synthesis and altered membrane functions in tumors.[41] Hence, inhibition of key enzymes in the lipid synthesis pathway (e.g., FASN) reduces cancer pathogenicity, and targeting this pathway may be useful in the development of novel cancer therapeutics.[46] Cancer cells depend on *de novo* lipogenesis for cell growth but there is evidence that dietary fat intake is linked to cell proliferation in breast cancer because breast cancer cells have the ability to acquire fatty acids from the circulation through lipoprotein lipase-mediated lipolysis.[48,49] While *de novo* lipogenesis is important for cancer cell growth, upregulated lipolysis—perhaps paradoxically at first glance—is another characteristic of cancer metabolism that is associated with disease aggressiveness. A key enzyme in cancer cell lipolysis is monoacyclglyerol lipase (MAGL).[50] Lipolysis catalyzed by MAGL not only increases the intracellular fatty acid pool but also leads to a diversion of fatty acids into the synthesis of oncogenic lipid messengers such as lysophospholipids, LPA, and eicosanoids.[50] Pharmacological inhibition of MAGL was therefore investigated in cancer therapy research and was found to decrease lipid messenger production and the metastatic potential of cancer cells. Alkylglyceronephosphate synthase (AGPS) was recently described as another candidate pharmacological target for breast cancer therapy. Like MAGL, this enzyme is upregulated in aggressive tumors, including those in breast cancer, and increases cell motility and invasion

through increased production of oncogenic lipid messengers.[51] Hence, both MAGL and AGPS modulate fatty acid utilization in breast cancer to favor synthesis of prometastatic signal molecules.

The level of phospholipid metabolism products is associated with the tumor ER status. Although levels of phospholipids (e.g., phosphatidylinositols, phosphatidylethanolamines, phosphatidylcholines) are increased in breast tumors in general, their content is significantly higher in ER-negative than ER-positive tumors and is positively associated with survival.[24] Metabolism of the membrane phospholipids phosphatidylcholine (PC) has been studied extensively in breast cancer biology.[52–54] The activity of the enzyme glycerol-3-phosphate acyltransferase is closely associated with tumor phospholipid levels.[55] The tumor content of PC and its precursors shifts when tumors respond to therapy, and these changes may have prognostic value in patients receiving chemotherapy.[56,57] The PC content may also indicate intrinsic disease aggressiveness, and it was shown that the glycerophosphocholine to phosphocholine ratio is higher in basal-like and luminal B breast tumors with poor prognosis than in luminal A-type tumors.[22,58] Previous studies have revealed that the breakdown of PC is profoundly altered in breast cancer; the level of phosphocholine and total choline-containing phospholipids increases with cell transformation, leading to a glycerophosphocholine to phosphocholine switch during the immortalization of cell lines.[52–54] Enhanced choline transport and increased synthesis of phosphocholine by choline kinase α, a known oncogene that is upregulated in breast cancer, together with alterations in phospholipase activities, have been identified as the root cause of these observations. However, it remains to be seen whether this switch in choline metabolism is truly a driver of disease aggressiveness or rather a marker for transformation. On the other hand, there is substantial evidence that the biologically active lipid messenger sphinosine 1-phosphate (S1P) contributes to disease progression in breast and other cancers.[59,60] S1P is a product of phosphorylation of sphingosine by sphingosine kinase 1. Sphingosine is a proapoptotic molecule, therefore it has been hypothesized that the conversion of sphingosine into S1P is oncogenic and enhances cancer cell survival.[59] Consistent with this hypothesis, pharmacological inhibition of sphingosine kinase 1 reduced metastasis in the 4T1 murine breast cancer model.[60] Together, these examples show that breast tumors undergo broad changes in lipid metabolism that balance the need for increased lipogenesis to maintain cancer cell growth with the need for lipolysis to maintain the supply of prosurvival and prometastatic lipid messengers. These alterations create metabolic dependencies of tumors. For this reason, enzymes involved in both lipogenesis and lipolysis are valid pharmacological targets in breast cancer therapy. Alternatively, inhibitors of lipid messenger signaling may be used to treat patients because breast cancer metastasis is probably susceptible to small molecule-based receptor antagonists that block receptor activation by these messengers, as has been shown for LPA and S1P signaling.[15,60]

Metabolic Profiles of the Molecular Subtypes in Breast Cancer

Breast cancer is a heterogeneous disease and breast tumors can be classified into several molecular subtypes with specific gene expression profiles.[61,62] These molecular subtypes also show differences in their mutational spectra and DNA methylation patterns,[63,64] indicating that they are distinct biological entities whose response to therapy is very different. Of all subtypes, basal-like tumors and HER2-positive, ER-negative tumors tend to produce the most aggressive disease.[65,66] Basal-like tumors overlap largely with a group of tumors referred to as triple-negative, meaning they are negative for ER, HER2, and progesterone receptor expression.[67] Recent metabolome studies have assessed the association of tumor metabolome patterns with these molecular subtypes and disease outcome. Whereas most ER-negative tumors are separated from ER-positive tumors on the basis of their markedly different metabolite abundance profiles,[1,2,24] further subclassification into HER2-positive and HER2-negative tumors was not achieved in a recent publication that used a compendium of 296 known metabolites for classification.[2] In the latter study, only luminal A tumors clearly separated from other molecular subtypes. This observation is in agreement with another study that observed a weak distinction between HER2-negative and HER2-positive tumors in an analysis of more than 500 lipids.[24] A third study reported separation of breast tissues into 2 clusters based on the relative abundance of 379 metabolites.[25] Cluster 1 contained mainly luminal-type tumors and non-cancerous tissues whereas cluster 2 was enriched for ER-negative tumors and metastatic lesions, yet a clear separation into molecular subtypes was not achieved. Very similar findings were obtained by another group.[3] In this study, an incomplete separation of normal tissues and ER-positive tumors was observed, and some of the ER-positive tumors clustered with ER-negative tumors, indicating overlapping phenotypes among these tumors based on metabolite abundance patterns. Since molecular subtypes defined by gene expression were not separated on the basis on their metabolism in these studies, it is possible that the tumor metabolome describes disease traits that are somewhat different from those captured by gene expression analysis. Alternatively, perhaps a different compendium of metabolites must be profiled to achieve a metabolite-based classification that matches the gene expression-defined subtypes. Nonetheless, like gene signatures, metabolite patterns may predict disease outcomes. Both Hilvo et al. and Terunuma et al. observed that tumor metabolite levels are candidate prognostic markers, while another research study showed that metabolite profiles in serum samples from breast cancer patients can predict which patients might experience early disease recurrence among surgically treated patients.[2,24,68] Thus, large-scale assessment of metabolite patterns in tumor and blood samples may assist in determining the aggressiveness of breast tumors at the time of diagnosis.

Links Between Tumor Metabolism and the Mutational Landscape

Although breast tumors acquire various mutations, somatic mutations in genes directly linked to metabolism are rare.[63] For example, only one study reported a mutation in the isocitrate dehydrogenase 1 or 2 genes.[69] Cancer genes with the highest mutation frequency include *TP53* and the PI3K subunit encoded by *PIK3CA*, both of which are mutated in 35–40% of all breast tumors.[63] *TP53* mutations are a dominant feature of the HER2-enriched and basal-like molecular subtypes, in which 70–80% of tumors harbor this mutation, indicating a close relationship between loss of TP53 function and development of these subtypes. In contrast, mutations in genes that regulate the PI3K-Akt-mTOR pathway (e.g., *PIK3CA*, *PIK3R1*, *PTEN*, *AKT1*) are more often found in luminal-type tumors (40–50% have these mutations) and support constitutive activation of this pathway. Several studies have investigated the effect of a *TP53* mutation on cancer metabolism. Wild-type TP53 regulates the balance between utilization of respiratory and glycolytic pathways, and loss of TP53 function leads to a metabolic switch toward glycolysis.[70] Expression of synthesis of cytochrome c oxidase-2 (*SCO2*), a target gene of wild-type TP53, enhances aerobic respiration whereas reduced expression of *SCO2* in the presence of mutant TP53 leads to reduced respiration and increased glycolysis. A second TP53 target gene, *TIGAR*, has another important role in this metabolic switch; TP53-inducible *TIGAR* inhibits glycolysis and decreases intracellular ROS while restraining autophagy.[71,72] In TP53 mutant/null cells, *TIGAR* expression is diminished while glycolysis and deleterious ROS production are increased. Mutant TP53 also increases the expression of mevalonate pathway genes and the flux through this pathway, leading to disruption of normal mammary tissue architecture and the acquisition of a cancer phenotype.[73] The same increased pathway flux regulates YAP/TAZ proto-oncogene function in mutant *TP53* cells.[74] In this context, mutant p53 acts as a positive transcriptional cofactor of oncogenic sterol regulatory element-binding proteins (SREBPs), leading to SREBP-induced mevalonate synthesis and mevalonate-dependent nuclear accumulation of YAP/TAZ, which subsequently increases cancer cell proliferation.[74] In turn, sterol biosynthesis intermediates were found to mimic the effects of mutant TP53 whereas treatment with a statin, Simvastatin, could reverse them.[73] These findings are notable because statins were predicted to be effective agents in the treatment of basal-like breast tumors,[75] which commonly harbor *TP53* mutations. Statin-based inhibition of sterol synthesis could therefore be effective not only in treating these aggressive *TP53*-mutant tumors but also in the therapy of luminal breast cancers in which sterol synthesis is a key source of the ER ligand 27HC, as mentioned earlier in this review. Experiments based on cell culture and animal models further showed that wild-type TP53 inhibits the amino acid-sensing mTOR complex 1 (mTORC1) and the monocarboxylate transporter 1 (MCT1) while regulating the SREBP-mevalonate axis.[76] As a result, wild-type TP53 inhibits protein synthesis and growth, reduces glycolysis by preventing lactate efflux, and exerts a generally antilipogenic effect on cells. Lastly, a specific connection between the tumor TP53 status and tumor glycerophospholipid levels was shown to exist. A joint analysis of metabolomics and genetics in human breast tumors revealed that tumors with *TP53* mutations have strongly reduced levels of certain phospholipids.[3]

Some of the effects of constitutive activation of the PI3K-Akt-mTOR pathway as a result of mutations of *PIK3CA* or other genes in this pathway on cancer metabolism are the same as those caused by the frequent loss of TP53 function in HER2-enriched and basal-like tumors. In contrast to *TP53* mutations, however, *PIK3CA* mutations predict good, rather than poor, survival.[77,78] PI3K-Akt-mTOR signaling has a decisive antiapoptotic effect and stimulates glycolysis, activates mTORC1 and protein synthesis, and enhances lipid production and lactate efflux.[79,80] Akt stimulates glycolysis through upregulation of mitochondrial hexokinase 2 and mTORC1-mediated effects on HIFα expression, and increases fatty acid synthesis through inhibition of glycogen synthase kinase 3β (GSK3β), leading to increased SREBP1 stability and lipid metabolism. Moreover, loss of the Akt signal was found to suppress oncogenesis in an mTORC1-dependent manner.[81] Because aberrant PI3K-Akt-mTOR pathway activation is so common in human breast tumors, pathway inhibitors have been developed for cancer therapy. These inhibitors alter tumor metabolism. A reduction in lactate and an increase in phosphocholine levels were described as biomarkers for response to PI3K inhibition in basal-like breast cancer.[82] However, a certain heterogeneity in pathway activation caused by *PIK3CA* mutations has been reported for human breast tumors. These mutations are commonly associated with a gene signature of PI3K-Akt pathway activation but may not be associated with an increase in mTORC1 signaling.[78] They also promote cancer by Akt-independent mechanisms.[83] Nevertheless, *PIK3CA* mutant cells are sensitive to mTOR inhibitory drugs such as the dual PI3K/mTOR inhibitor NVP-BEZ235.

The amplification of distinct genomic regions is another hallmark of breast cancer and causes overexpression of several key oncogenes including the *ERBB2* gene encoding the HER2 receptor. In basal-like breast cancer, the *AKT1*, *PIK3CA*, and *MYC* loci are commonly amplified and induce constitutive Akt and c-Myc pathway signaling.[63,84] c-Myc activation has a particularly strong effect on the cancer metabolome, partly because c-Myc stimulates mitochondrial biogenesis.[85] This function of c-Myc may explain why the metabolic profile of ER-negative breast cancer describes tumors with a c-Myc activation signature as a distinct disease subtype, as shown recently by Terunuma et al.[2] c-Myc expression leads to metabolic reprogramming and oncogenic stress in tumors, including glutamine addiction. Hence, aberrant c-Myc activation may lead to metabolic dependencies that provide tumor-specific targets for pharmacologic intervention.[86] Targets in c-Myc-driven tumors include glucose and glutamine transporters, lactate dehydrogenase A, serine hydroxymethyltransferase, and mitochondrial glutaminase. To this end, glutaminase inhibitors such as CB-839[29] may impair the growth of tumors with constitutive c-Myc activation to a greater extent

than other tumors because of their dependency on exogenous glutamine.

Metabolic adaptation to stress

The metabolism of tumors evolves with disease progression and undergoes significant changes during adaptation to the stress that arises with oncogene addiction, hypoxia, metastasis, or cancer therapy. For example, mitochondrial ROS were shown to be essential for K-Ras-induced tumorigenesis.[87] In pancreatic cancer, this increased ROS production is counterbalanced by a K-RAS–regulated metabolic pathway, in which glutamine-derived aspartate is transported into the cytoplasm where it is converted into malate and pyruvate to increase NADPH and maintain the cellular redox state.[88]This adaptation renders pancreatic cancer cells susceptible to inhibitors of glutamic-oxaloacetic transaminases (GOT). Other oncogenes, including MYC, also increase ROS production; these ROS, such as hydrogen peroxide, stimulate cancer cell proliferation.[89] Malignancies in which dominant oncogenes facilitate growth are dependent on increased ROS for both anchorage-independent survival and proliferation. This increased ROS generation was shown to be fueled by mitochondrial glutamine catabolism.[87] Without ROS, K-Ras–mediated tumorigenesis is blocked, indicating that inhibitors of either mitochondrial function, glutamine catabolism, or downstream targets of ROS, such as the ERK-MAPK signaling pathway, may have antitumor effects in these oncogene-addicted tumors. Cell survival during energy stress is further maintained through activation of the AMP-activated protein kinase (AMPK) pathway, which enhances NADPH generation in primary tumors through increased fatty acid oxidation.[90] However, a bioenergetic adaptation occurs when breast tumors become metastatic.[91,92] As a result, metastatic cells decrease their proliferation rate because they metabolize NADPH to detoxified ROS instead of using it for fatty acid synthesis and cell growth. It has been argued that this altered consumption of NADPH cannot support both rapid growth and protection against oxidative damage during metastasis, leading to a shift of NADPH consumption into pathways such as glutathione synthesis that counteract oxidative stress.[91] Accordingly, in late-stage breast tumors and metastatic lesions to the brain, activity of the pentose phosphate pathway, glycolysis, and the TCA cycle is increased but their products are diverted into ROS detoxification rather than fatty acid or nucleotide synthesis.[91,92] This reprogramming can reduce the susceptibility of metastatic disease to standard cancer therapies whose cytotoxicity is interlinked with their ability to elicit oxidative stress, such as chemotherapeutics (e.g., doxorubicin), radiation, proteosome inhibitors (e.g., PS341), or agents that induce glucose deprivation (e.g., 2-deoxy-D-glucose).[92] A common stress factor in cancer is the hypoxia that develops when tumor growth exceeds blood supply. Hypoxia is a driver of cancer progression and triggers significant changes in cancer metabolism. Oxygen deficiency directly affects mitochondrial function but also alters the expression of many genes including hypoxia inducible factor 1 (HIF1α), the key mediator of the hypoxia response.[93] Hypoxia induces glycolysis and reductive carboxylation of glutamine, thus maintaining mitochondrial citrate synthesis under oxygen deficiency.[32] Although glycolysis is needed to maintain ATP synthesis in the absence of mitochondrial respiration, some of the endproducts of glycolysis, like lactate and ketones, are now known to promote metastasis in breast cancer. Lactate production is required for tumor progression and metastasis in animal models and elevated tumor lactate levels predict poor survival in breast and other cancers.[94–97] The two lactate dehydrogenase isoforms, A and B, are both candidate targets for breast cancer therapy.[98] In addition, studies have shown that lactate drives tumor angiogenesis, and elevated lactate and ketone concentrations have been associated with the development of a cancer stem cell phenotype in breast cancer.[97,99] Thus, metabolic adaptation not only supports tumor growth, but also assures survival of metastatic lesions and can induce an aggressive phenotype such as increased metastatic potential and resistance to therapy.

Metabolic Biomarkers for Prediction of Cancer Progression and Therapy Response

The assessment of tissue and serum/plasma metabolic markers holds great promise for the discovery of biomarkers of disease progression and response to therapy.[100] Investigations of breast cancer metabolism showed marked differences between ER-negative and ER-positive tumors; in particular, glutaminolysis and the metabolism of certain lipids and serine are significantly higher in ER-negative tumors.[1,2,24–26,91] These differences may guide therapies that target metabolic pathways. Moreover, phospholipids including lysophosphatidylcholine and phosphatidylcholine have been described as markers for disease aggressiveness, response to therapy, and patient survival.[24,56,57,82,91,101] Other metabolism-based biomarkers for breast cancer include choline, lactate, and several amino acids.[52–54] These markers have been described by various groups that investigated either tumor biology or biochemical changes induced by cancer therapeutics to test whether these changes can be used to develop predictive biomarkers. Such research showed that lactate may have an important role in taxol resistance and is also a marker of response to PI3K inhibition in basal-like breast cancer.[82,102] Likewise, metabolites in the pyrimidine pathway may predict tamoxifen resistance in breast cancer,[103] and inhibition of this pathway may sensitize patients to tamoxifen. Others reported that a combination of 11 blood-based metabolite markers can be used in a noninvasive test to forecast disease recurrence in breast cancer, outperforming the current clinical marker, CA 27.29.[68] The same group also showed that the abundance of 4 metabolites, mainly amino acids, in breast tumors can predict pathological response to treatment with neoadjuvant chemotherapy.[104] Although most of these observations require independent validation in larger patient cohorts, the described nuclear magnetic resonance spectrometry- and mass spectrometry-based approaches will undoubtedly advance the field of tissue and serum/plasma metabolomics and may lead to the development of robust prognostic and predictive tests (**Fig. 2**). Finally, it should be mentioned that resistance to therapy can be induced by metabolites

that originate from bystander cells. As one example, it was recently shown that tumor-infiltrating mesenchymal stem cells induce resistance to a cisplatin-based chemotherapy through the release of fatty acids.[105] Future investigations into the association of tissue and serum metabolites with disease outcome may have great implications in cancer research.

Concluding Remarks

Advances in our knowledge of breast cancer metabolism have led to the discovery of metabolic alterations and dependencies that can serve as candidate biomarkers of diagnosis, prognosis, and clinical intervention. However, most of the biomarker studies completed to date have generated preliminary observations that require validation in larger patient cohorts. Future research should focus on the metabolic profiling of blood and urine samples that have been obtained from patients prior to disease diagnosis, thus applying a noninvasive approach to identifying early disease markers. Other efforts should evaluate the metabolome in biofluids and tumors from patients at diagnosis to identify prognostic metabolites that predict metastasis and survival. These efforts should use integrated analysis of metabolome, transcriptome, and genome level data to allow improved characterization of the disease. The approach will require well-selected patient pools and independent validation in adequately powered studies. Equally important is the metabolic profiling of patients before and after therapy. This research should yield predictive markers for therapy response and also allow assessment of post-therapy metabolism for future treatment opportunities. Finally, agents targeting metabolic pathways and metabolism-driven signal transduction pathways should be brought into the clinic because they have potential to achieve therapeutic responses in tumors with oncogene addiction (e.g., Myc- and Ras-driven tumors) that cannot be achieved by current standard therapies. Currently, most strategies to target metabolic enzymes for cancer therapy have only been evaluated in preclinical models. The transition into clinical trials is slow even though targeting enzymes in the nucleic acid synthesis pathway has historically led to the development of several approved anticancer drugs that are now widely used.[8] In the case of breast cancer, the glutaminase inhibitor CB-839[29] will be evaluated in patients with triple-negative breast cancer (ClinicalTrials.gov Identifier NCT02071862), and one can only hope that other candidate drugs that inhibit cancer metabolism will soon follow this path.

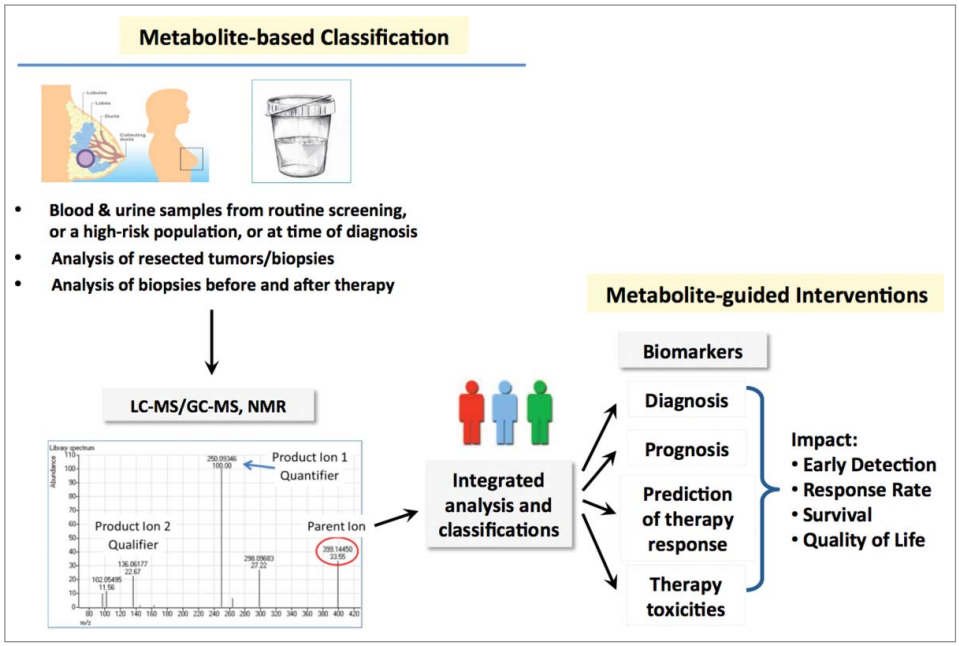

Figure 2. Metabolic profiling to guide early detection, prognosis, and response to therapy. Various approaches are being applied to develop metabolite-based tests for early disease detection and stratification of patients for clinical management of the disease. LC-MS: liquid chromatography–mass spectrometry; GC-MS: gas chromatography–mass spectrometry; NMR: nuclear magnetic resonance spectroscopy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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