

## Review

## Crosstalk between Estrogen Signaling and Breast Cancer Metabolism

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**Estrogens and estrogen receptors (ERs) regulate metabolism in both normal physiology and in disease. The metabolic characteristics of intrinsic breast cancer subtypes change based on their ER expression. Crosstalk between estrogen signaling elements and several key metabolic regulators alters metabolism in breast cancer cells, and enables tumors to rewire their metabolism to adapt to poor perfusion, transient nutrient deprivation, and increased acidity. This leads to the selection of drug-resistant and metastatic clones. In this review we discuss studies revealing the role of estrogen signaling elements in drug resistance development and metabolic adaptation during breast cancer progression.**

Estrogens and ERs regulate metabolism in both normal physiology and in disease. Despite lack of sexual reproduction, invertebrates possess ER-like proteins that regulate their metabolic processes, demonstrating the importance of ER signaling elements for non-reproductive functions including metabolism [1]. Metabolic changes in postmenopausal women after estrogen loss suggest that estrogen signaling regulates energy metabolism [2]. Nutrient availability is one of the important parameters for cell-cycle progression and proper energy metabolism. Thus, cells constantly check the amount of nutrients in their microenvironment. Because cancer cells proliferate in an uncontrolled way, they develop different strategies to keep their proliferation rate stable even under limited nutrient conditions. This concept is called metabolic plasticity. In 2011, Hanahan and Weinberg updated their milestone review to include metabolic plasticity as an emerging cancer hallmark, called 'deregulation of cellular energetics' [3]. Tumors reprogram their metabolism to adapt to poor perfusion (lack of sufficient blood supply), leading to transient nutrient deprivation and increased acidity. During adaptation to environmental stress, drug-resistant and metastatic clones are selected [4,5]. In this review we focus on the impact of estrogen signaling elements on metabolic alterations taking place during breast cancer progression. We caution the readers that the majority of studies reported here are fairly recent and still require rigorous validation, but they contribute to a comprehensive framework that might explain some of the clinical observations regarding ER<sup>+</sup> tumors.

### Metabolic Differences between Breast Cancer Cell Subtypes

There are four intrinsic molecular subtypes of breast cancer: luminal A, luminal B, HER2<sup>+</sup>, and triple-negative breast cancer (TNBC) [6]. Each tumor subtype has a distinct proliferation rate, metastatic capacity, and metabolic phenotype and genotype (Boxes 1 and 2 and Table 1). Transcriptomics combined with metabolomic analyses have identified altered metabolite levels and associated metabolic pathways in different subtypes.

### Glucose-Metabolizing Pathways

In a recent study, tumor samples across 33 different cancer types were analyzed and categorized based on the mRNA expression of seven major metabolic pathways. Patients with tumors that

### Highlights

Different breast cancer subtypes have different metabolic phenotypes.

ERs modulate the expression of genes important for metabolic regulation.

Extranuclear ERs regulate kinase signaling pathways as well as mitochondrial metabolism to modulate cancer cell metabolism.

Metabolic rewiring is an inherent property of endocrine resistance, but it is not clear whether it is a driver or consequence of endocrine resistance.

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### Box 1. Molecular Subtypes of Breast Cancer

Breast cancer classification and patient stratification is crucial in terms of determining treatment strategy in clinic. Breast tumors are classified into two groups: *in situ* (20% of all cases) and invasive breast tumors (80% of all cases). Based on their location, *in situ* breast cancers are further classified into two groups: ductal carcinoma *in situ* (DCIS, ~80% of *in situ* breast cancers) and lobular carcinoma *in situ* (LCIS, ~20% of *in situ* breast cancers). About 20–50% of DCIS tumors can eventually progress to an invasive carcinoma [126]. Invasive breast cancers have the capacity to spread to other organs in the body. Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) expression is used in the clinic to determine breast cancer subtype. In addition, expression levels of Ki-67 are also utilized to decide treatment options for patients. Based on expression of these biomarkers, invasive breast tumors are divided into four main molecular subtypes of breast cancer: luminal A, luminal B, HER2-enriched, and triple-negative (TNBC)/basal-like [127,128]. The luminal A subtype is hormone receptor-positive (HR<sup>+</sup>), HER2-negative (HER2<sup>-</sup>), and has a low Ki-67 signature. It has relatively better prognosis and can be managed by endocrine therapies. The luminal B subtype is also HR<sup>+</sup> but it can be either HER2<sup>+</sup> or HER2<sup>-</sup>, with higher Ki-67 expression and relatively worse prognosis and less response to endocrine agents. The HER2-enriched subtype is HR<sup>-</sup> and HER2<sup>+</sup>, and has a worse prognosis compared to the luminal subtypes. It is treated with HER2-targeting agents in the clinic. TNBC/basal-like subtype has no hormone receptor or HER2 expression, and is more common in African-American women and in individuals with *BRCA1* mutation [128]. Overall, this is the most aggressive subtype and has limited targeted therapy options in the clinic. Clinical studies showed that >75% of all breast cancers express ER and/or PR, and ~10–15% also express HER2 [129].

### Box 2. Metabolic Genes Mutated in Different Breast Cancer Subtypes

Mutation profiles of metabolic genes are different in different breast cancer subtypes. In general, *TP53* and *MYC* mutations are most commonly found in HER2<sup>+</sup> and basal-like/TNBC subtypes [10,127], whereas 40–50% of luminal-type tumors have mutations in PI3K/AKT/mTOR pathway elements (e.g., *PIK3CA*, *PIK3R1*, *PTEN*, *AKT1*) [128]. *PIK3CA* and *MAP3K1* are the most frequently mutated genes in this subtype [129]. On the other hand, glycolytic enzymes (e.g., *HK3*, *GPI*, *GAPDH*, *PGK1*, *ENO1*), glycolysis regulator (*PDK1*), and pentose phosphate pathway enzymes (*PGD*, *TKT*, *RPIA*) are the main metabolic genes with mutations in ER<sup>-</sup> breast cancers [130]. Lipid metabolism-related genes (e.g., *CPT-1A* and *FASN*) are upregulated in the HER2<sup>+</sup> subtype, whereas *IDH1* [131] and *AKT3* mutations are the most common in the basal-like/TNBC [132] subtype.

have upregulated carbohydrate, nucleotide, and vitamin/cofactor metabolism had a worse prognosis, while those with upregulated lipid metabolism had a better prognosis [7]. Additional studies showed that ribulose 5-phosphate, fumarate, 2-hydroxyglutarate (2-HG), glutamate/glutamine ratio, serine metabolites, kynurenine, monoacylglycerols (MAG), and most phospholipid and sphingolipids are increased in the TNBC subtype versus the luminal subtype [8–10]. Consistent with these results, transporters involved in macronutrient uptake and metabolic enzymes, such as GLUT1, SLC1A5, SLC7A5, GLS1, and PGDH, are upregulated in TNBC [11–13]. Because preclinical studies suggest that TNBC relies more on the glucose metabolism, this also creates a metabolic vulnerability that can potentially be targeted by metformin [14,15]. Similarly, pentose phosphate pathway intermediates, such as G6PDH and 6PGL, are also upregulated in HER2<sup>+</sup> and TNBC subtypes compared to luminal subtypes [16,17]. Another recent study identified reduced tricarboxylic acid (TCA) cycle activity and oxidative phosphorylation (OXPHOS) via glycolysis pathways in luminal A invasive lobular carcinoma subtype of tumors compared to the luminal A invasive ductal carcinoma subtype of tumors [18].

### Amino Acid-Metabolizing Pathways

Amino acid metabolism is also different in different breast cancer subtypes (Table 1). *MYC* amplification is the main regulator of glutamine-related metabolic rewiring. *MYC* facilitates excess glutamine uptake by inducing the expression of glutamine transporters and glutamine-metabolizing enzymes in breast cancers [19]. This molecular mechanism is upregulated in the luminal B, TNBC, and HER2<sup>+</sup> subtypes relative to luminal A subtypes [20]. Interestingly, inhibition of ASCT2/SLC1A5-mediated transport with pharmacological inhibitors reduces glutamine uptake only in TNBCs and not in luminal subtypes [21].

Table 1. Molecular Differences in Different Breast Cancer Subtypes.

Breast cancer subtypes and metabolic characteristics			
Luminal A subtype	Luminal B subtype	HER2 <sup>+</sup> subtype	Basal-like/TNBC
Glucose metabolism			
		G6PDH and 6PGL are upregulated in HER2 <sup>+</sup> subtypes relative to luminal subtypes [16,17]	Ribulose 5-phosphate, fumarate, 2-HG, glutamate/ glutamine ratio, serine metabolites, kynurenine, and MAG are elevated in the TNBC subtype relative to the luminal subtype [7] GLUT1, SLC1A5, SLC7A5, GLS1, and PGDH are upregulated in TNBC [11–13] G6PDH and 6PGL are upregulated in TNBC subtypes relative to the luminal subtypes [16,17]
Amino acid metabolism			
	MYC is overexpressed in luminal B versus luminal A [19]	MYC is overexpressed in HER2 <sup>+</sup> versus luminal A [19] MYC-related serine, glycine, and tryptophan uptake and the synthesis of one-carbon units result in a more active TCA cycle in HER2 <sup>+</sup> tumor cells [8,21]	MYC is overexpressed in luminal B, HER2 <sup>+</sup> , and TNBC relative to luminal A [19] Inhibition of ASCT2/SLC1A5-mediated glutamine uptake is more effective in TNBC than in luminal subtypes [20] MYC-related serine, glycine, and tryptophan uptake and synthesis of one-carbon units result in a more active TCA cycle in TNBC [8,21] The enzymes responsible for serine/glycine biosynthesis, such as PGDH, PSP, and SHMT, are expressed at higher levels in ER <sup>-</sup> subtypes than in ER <sup>+</sup> subtypes [13,22]
Fatty acid metabolism			
Inhibition of 27-hydroxycholesterol synthesis decreases cell proliferation in ER <sup>+</sup> cancers but not in ER <sup>-</sup> cancers [27,28] <sup>a</sup>	Inhibition of 27-hydroxycholesterol synthesis decreases cell proliferation in ER <sup>+</sup> cancers but not in ER <sup>-</sup> cancers [27,28] <sup>a</sup>	The expression of PLIN1, CPT-A1, ACLY, SCD1, and FASN is highest in the HER2 <sup>+</sup> subtype [24,25] Phospholipid and sphingolipid levels are high in ER <sup>-</sup> breast cancers [7,29,30] <sup>a</sup>	PLIN1, CPT-A1, ACLY, and FASN expression is lowest in the TNBC subtype [24,25]. Phospholipid and sphingolipid levels are high in ER <sup>-</sup> breast cancers [7,29,30] <sup>a</sup>
Macronutrient metabolism			
High vitamin B levels lowered the risk of breast cancers in all subtypes [23] Folate has a protective effect	High vitamin B levels lowered the risk of breast cancers in all subtypes [23] Folate has a protective effect	High vitamin B levels lowered the risk of breast cancers in all subtypes [23] Thiamine is protective	High vitamin B levels lowered the risk of breast cancers in all subtypes [23]

Table 1. (continued)

Breast cancer subtypes and metabolic characteristics			
against ER <sup>+</sup> PR <sup>+</sup> HER2 <sup>-</sup> subtypes [23] <sup>a</sup>	against ER <sup>+</sup> PR <sup>+</sup> HER2 <sup>-</sup> subtypes [23] <sup>a</sup>	against HER2 <sup>+</sup> subtypes [23]	
Metabolic gene mutations			
<i>PIK3CA</i> , <i>PIK3R1</i> , <i>PTEN</i> , <i>AKT1</i> , <i>MAP3K1</i> [128,129]	<i>PIK3CA</i> , <i>PIK3R1</i> , <i>PTEN</i> , <i>AKT1</i> , <i>MAP3K1</i> [128,129]	<i>TP53</i> , <i>MYC</i> [10,127] <i>HK3</i> , <i>GPI</i> , <i>GAPDH</i> , <i>PGK1</i> , <i>ENO1</i> , <i>PDK1</i> , <i>PGD</i> , <i>TKT</i> , <i>RPIA</i> [130] <i>CPT-A1</i> , <i>FASN</i> [26]	<i>TP53</i> , <i>MYC</i> [10,127] <i>HK3</i> , <i>GPI</i> , <i>GAPDH</i> , <i>PGK1</i> , <i>ENO1</i> , <i>PDK1</i> , <i>PGD</i> , <i>TKT</i> , <i>RPIA</i> [130] <i>IDH1</i> , <i>AKT3</i> [131,132]

<sup>a</sup>ER<sup>+</sup> and PR<sup>+</sup> are present in luminal A and luminal B subtypes, but not in HER2<sup>+</sup> and TNBC subtypes.

MYC also upregulates serine, glycine, and tryptophan uptake and the synthesis of one-carbon units, resulting in a more active TCA cycle in HER2<sup>+</sup> and TNBC breast cancer subtypes [8,22]. Functional genomic studies revealed that the serine synthesis pathway is essential for breast cancer cells, and the enzymes responsible for serine and glycine biosynthesis, such as PGDH, PSP, and SHMT, are expressed in ER<sup>-</sup> breast cancers more than in ER<sup>+</sup> [13,23]. Cancarini *et al.* demonstrated the impact of one-carbon metabolism-associated vitamins in different breast cancer subtypes. Overall, high vitamin B intake lowered breast cancer risk in all subtypes. Notably, folate has a protective effect against ER<sup>+</sup> PR<sup>+</sup> and HER2<sup>-</sup> breast cancers whereas thiamine is protective against HER2<sup>+</sup> breast cancers [24].

### Lipid-Metabolizing Pathways

Owing to high metabolic demand, lipid provision is essential for cancer cells. Because lipid metabolism is directly related to intrinsic tumor biology and the tumor microenvironment, genes associated with lipid metabolism are differentially expressed in different subtypes. Kim *et al.* showed that, whereas HER2<sup>+</sup> subtypes have the highest expression levels of *PLIN1*, *CPT-A1*, and *FASN*, TNBC subtype tumors have the lowest expression of these genes [25]. Similarly, *ACLY*, *FASN*, and *SCD1* are overexpressed in the HER2<sup>+</sup> subtype, whereas TNBC subtypes express less *ACLY*, suggesting that *de novo* lipid synthesis is upregulated in the HER2<sup>+</sup> subtype [26]. However, there are contradictory studies suggesting that *FASN* overexpression is also a targetable therapy option for TNBCs [27].

In addition to lipid metabolism-related enzymes, the levels of lipid metabolites also differ depending on the subtype. Several groups reported that inhibition of 27-hydroxycholesterol synthesis decreases the proliferation of ER<sup>+</sup> breast cancers, but not of ER<sup>-</sup> cancers [28,29]. Furthermore, several studies showed that various phospholipids and sphingolipids are upregulated in ER<sup>-</sup> subtypes relative to ER<sup>+</sup>, and higher phospholipid concentrations correlated with high tumor grade [30,31]. Overall, these studies indicated that the TNBC and HER2<sup>+</sup> subtypes adapt to a more active metabolic phenotype compared to the luminal subtypes to sustain high metabolic demand during rapid cell proliferation (Table 1).

### Crosstalk between Macronutrient Metabolism and Estrogen Signaling

ERs regulate expression and activity of many enzymes involved in metabolic pathways (Figure 1).

### Regulation of Glucose Metabolism

In cancer cells, key glucose transporters and glycolytic enzymes are upregulated to support the synthesis of the building blocks required for cell proliferation, causing a metabolic shift towards glycolysis, which is known as the 'Warburg effect' [32–34]. Hypoxia-dependent upregulation of ER signaling stimulates the expression of glucose transporters (GLUT-1, GLUT-2, and GLUT-5) in breast cancer cells [33]. MCF-7 cells switch their metabolic pathways in response to 17 $\beta$ -estradiol (E2) and glucose availability. A high glucose level in the media activates AKT signaling and suppresses the TCA cycle [35]. E2 treatment upregulates the *c-Myc-hnRNP* axis and the expression and activity of glycolytic enzymes, such as PFKFB3, resulting in increased levels of fructose 2,6-bisphosphate (F26BP) and glucose uptake [36,37]. In breast cancer cells, E2 regulates the balance between glycolysis and OXPHOS by upregulating PDH in the absence of glucose [38]. E2 also promotes addiction to the pentose phosphate pathway by upregulating G6PD enzyme in breast cancer cells [38]. Lactate production is also upregulated by E2 in breast cancer cells [39]. Expression of HK and PFK also positively correlated with ER expression in breast carcinomas, and inhibition of HK results in high toxicity in breast cancer cells [40–42]. In addition to the two classic ERs, GPER-1 (the membrane G protein-coupled ER) has been shown to control estrogen-mediated angiogenesis by upregulating PFKFB3 [43].

### Regulation of Amino Acid Metabolism

Glutamine is the most abundant amino acid in the serum and is preferentially consumed by breast tumors in an ER-dependent manner [11,44]. Although a limited number of studies have demonstrated crosstalk between estrogen signaling and amino acid metabolism, clinical studies showed that ER<sup>+</sup> tumors were glutamine-enriched and glutamate-reduced relative to ER<sup>-</sup> breast tumors [11,12,45]. Specifically, the enzymes responsible for glutamine synthesis (GS, GDH) are upregulated in ER<sup>+</sup> breast cancer cells relative to ER<sup>-</sup> cells [46,47]. Conversely, glutamine uptake and enzymes related to glutamine catabolism (GLS) were shown to be upregulated in ER<sup>-</sup> breast tumors [48]. Accordingly, glutaminase inhibitor treatment was reported to have a better antitumor effects on ER<sup>-</sup> breast cancer cells [49]. In addition to glutamine, dietary arginine supplementation and upregulated ASL levels are associated with a high cell proliferation rate in breast tumors, and inhibition of ASL activity was shown to inhibit ER<sup>-</sup> breast cancer growth [50]. Asparagine is directed to the TCA cycle during adaptation to glutamine depletion in metastatic ER<sup>-</sup> murine breast cancer cells [51,52]. Leucine deprivation inhibits cell proliferation and induces apoptosis in ER<sup>+</sup> breast cancers by decreasing FASN expression [53]. These results suggest that there is complex crosstalk between estrogen signaling and amino acid synthesis pathways.

### Regulation of Lipid Metabolism

Clinical observations showed that higher levels of E2 lead to decreased fat accumulation, whereas ovariectomy, antiestrogen therapy, and menopause have the inverse effect in females [54]. Both E2 treatment and ER $\alpha$ /ER $\beta$  overexpression suppress lipogenesis and triglyceride accumulation via competitive binding to PPAR $\gamma$  in adipose tissue and various hormone-related cancers [55–57]. These effects are due to upregulation of leptin (*LEP*) and *STAT3* genes in the liver, and of *XBP* in endocrine-related cancers [58–60]. In addition, estradiol downregulates the expression of CD36, a transporter for free fatty acids, in breast cancer cells [61]. FASN and ACC-1 are overexpressed in breast cancer cells compared to normal cells, and their inhibition was proposed as a potential therapy in breast cancers [62,63]. Inhibition of SCD-1 was also shown to inhibit breast tumor growth [64]. In addition to changes in lipid metabolism pathways, specific free fatty acids were shown to impact on the energy status and cell viability of breast cancer cells. Anacardic acid, but not oleic acid or salicylic acid, inhibited cell viability in ER<sup>+</sup> cells by reducing cellular respiration [65]. Lastly, the biosynthesis of choline, which is a vitamin-like essential nutrient and an important element in the plasma membrane, was found to be regulated by ER $\alpha$  [66].

The effects of estrogen signaling on lipid metabolism are not unidirectional. Inhibition of FASN suppresses E2-stimulated cell growth and survival in breast cancer cells via activation of apoptotic pathways and inactivation of the oncogenic PI3K/AKT pathway [67]. Many groups have shown that 27-hydroxycholesterol can act as a ligand for ER and promote cell proliferation via ER signaling in breast cancers [28,29]. In conclusion, there is bidirectional signaling between estrogen signaling and lipid metabolism. More studies will be necessary to elucidate the molecular mechanism underlying this crosstalk.

### Crosstalk between Metabolic Regulators and Estrogen Signaling

ERs play a center role in metabolic regulation through cross-talk with various key regulators in the cell (Figure 2).

#### ER–Hypoxia-Inducible Factor (HIF) Crosstalk

HIF is a central regulator of oxygen homeostasis in the cell. As the tumor grows the tumor core becomes hypoxic owing to the diffusion limit of oxygen. This lack of oxygen activates a transcriptional complex involving HIF-1 $\alpha$ , thus deregulating numerous genes that are crucial for metabolic adaptation, angiogenesis, and eventually metastasis [68]. High HIF-1 $\alpha$  expression in tumors is associated with poor disease outcome and higher mortality in breast cancer patients [69]. Recently, HIF-1 $\alpha$  was shown to be a transcriptional target of ER $\alpha$  and high HIF-1 $\alpha$  expression was associated with tamoxifen resistance in ER $\alpha$ <sup>+</sup> tumors [70]. By contrast, the hypoxia response reduces ER $\alpha$  expression and cell proliferation [71]. Other studies showed that HIF-1 $\alpha$  and ER $\alpha$  transcriptionally regulate a common group of genes [72]. In addition to ER $\alpha$ , GPER-1, which mediates the non-genomic effects of estrogens, promotes HIF-dependent transcription and stimulates glycolysis in endocrine-regulated cells [72,73]. This well-established crosstalk enables new therapeutic approaches to treat hypoxic ER<sup>+</sup> breast cancers [71,74].

#### ER–Ras/Raf/MAPK Crosstalk

The Ras/Raf/MAPK pathway is one of the best-studied signal transduction pathways in the cell, and it acts as a highway for the intercommunication of the intracellular and extracellular environments. The oncogenic role of the Ras/Raf/MAPK pathway is very well studied in breast cancers; however, the role of ER $\alpha$  in this pathway remains elusive. Studies comparing the expression levels of the members of Ras/Raf/MAPK pathway in different breast cancer subtypes reported that patient samples with overexpression of Ras and MAPK proteins are more invasive and have lower ER $\alpha$  expression [75]. Consistent with these data, most transgenic *in vivo* models overexpressing Ras develop ER<sup>−</sup> breast tumors. Ando *et al.* recently developed an *in vivo* breast cancer mouse model expressing a constitutively active KRAS in mammary tissue. This model developed ER<sup>+</sup> adenocarcinomas with luminal A subtype breast cancer characteristics [76]. FASN activation by mutant KRAS was shown in lung cancer, and similar effects of Ras/MAPK pathway activation in ER<sup>+</sup> breast cancer remain to be explored [77].

#### ER–PI3K/AKT/mTOR Crosstalk

The PI3K/AKT/mTOR pathway is a key regulator of all cellular characteristics which are essential for the vitality of a cell, such as proliferation, metabolism, motility, survival, and apoptosis. Components of PI3K/AKT/mTOR pathway, including PIK3CA, PTEN, PIK3R1, and AKT1, are mutated in nearly 25% of breast tumors and are associated with drug responses in ER<sup>+</sup> and ER<sup>−</sup> tumors [78–80]. Estrogens stimulate this pathway to regulate the migratory and invasive characteristics of ER<sup>+</sup> tumors [81,82]. In return, mTOR signaling regulates the expression level and activity of ER $\alpha$ . mTOR also acts as a coregulator for ER $\alpha$  [83,84]. ER $\alpha$ –PI3K crosstalk has been studied intensively. In a recently identified mode of action, the PI3K pathway regulates ER-dependent transcription in breast cancer cells through the epigenetic regulator

KMT2D, and PI3K pathway inhibition activated the methyltransferase activity of KMT2D, leading to the activation of ER [85]. PI3K inhibition increases ER levels and activity [79,85], and PI3K inhibitor–endocrine agent combinations were tested with minor success in clinical trials to treat women with endocrine-resistant disease (NCT01339442, NCT02273973) [86,87]. Clinical trials are ongoing with next-generation PI3K inhibitors (NCT01971515, NCT03056755, NCT02684032, and NCT02437318) in advanced hormone receptor-positive breast cancers, and positive results were reported recently [88,89].

#### ER–p53 Crosstalk

p53 is the best-known tumor-suppressor protein in the cell. The p53 pathway responds to extracellular and intracellular stress conditions and controls cell-cycle checkpoints. ERs and p53 directly regulate the expression of each other. Further, E2-induced cell proliferation was shown to be regulated by p53 or via direct interaction between ER $\alpha$  homodimers and the p53 heterocomplex [90,91]. Recent studies showed that ER $\alpha$ /p53-mediated transcriptional regulation was attenuated by ER $\beta$  through a direct physical interaction between ER $\beta$  and p53 [92]. Somatic loss of ER $\beta$  accelerates the formation of p53-deficient mammary tumors, suggesting a protective role of ER $\beta$  against breast tumorigenesis [93].

#### ER–c-MYC Crosstalk

The c-MYC pathway regulates cell growth and proliferation. It was recently shown to orchestrate metabolic pathways which supply nutrients and other required elements to activate DNA replication and cell division. c-MYC is a proto-oncogene and a direct target and coregulator of ER $\alpha$  in breast cancer [94]. Upregulation of c-MYC and its downstream effectors is associated with poor disease outcome, high metastatic capacity, and endocrine resistance in breast tumors [95]. ER $\alpha$  and c-MYC act synergistically to induce cell proliferation [96,97]. A recent study found that ER and HER2<sup>+</sup> crosstalk regulates glutamine metabolism through c-MYC in aromatase inhibitor-resistant cells [98].

#### Metabolic Alterations in Endocrine-Resistant Breast Tumors

Selective estrogen receptor modulators (SERMs), downregulators (SERDs), and aromatase inhibitors are the first-in-line treatment strategy for ER<sup>+</sup> tumors [99]. However, one-third of all ER<sup>+</sup> breast tumors eventually develop resistance to these treatments. Drug-resistant and metastatic clones are selected as tumors reprogram their metabolism to adapt to poor perfusion, transient nutrient deprivation, and increased acidity. Identification of the crosstalk between ER signaling and endocrine resistance-driven metabolic alterations will be crucial to overcome resistance. Moreover, the distinct metabolic programs define metastatic organ sites for breast cancer cells; for example, activation of AKT and glycolytic pathways was seen in liver-specific metastases, emphasizing the importance of unique metabolic pathway adaptations in cancer cells [100].

Metabolic enzymes which facilitate reactions in the committed steps of glycolysis are dysregulated and are potential targets in recurrent breast tumors. PFKFB3 is an activator of PFK1, the key regulator of the second committed step in glycolysis. Several groups showed that targeting PFKFB3 may be a potential strategy to overcome endocrine resistance [43,101]. Inhibition of HK2, which facilitates the first committed step of glycolysis, is associated with retarded growth of tamoxifen-resistant breast tumors. HIF-1 $\alpha$  hyperactivation via modulation of the AKT/mTOR and/or AMPK signaling pathways contribute to tamoxifen resistance [102]. Of note, a novel epigenetic modulator, NSD2, is upregulated in tamoxifen-resistant cells, leading to methylation of the promoters of genes encoding key glucose metabolic enzymes such as HK2 and G6PDH as well as TIGAR [103]. These results reveal

that endocrine resistance is promoted by multiple mechanisms that induce glycolytic flux in breast cancers.

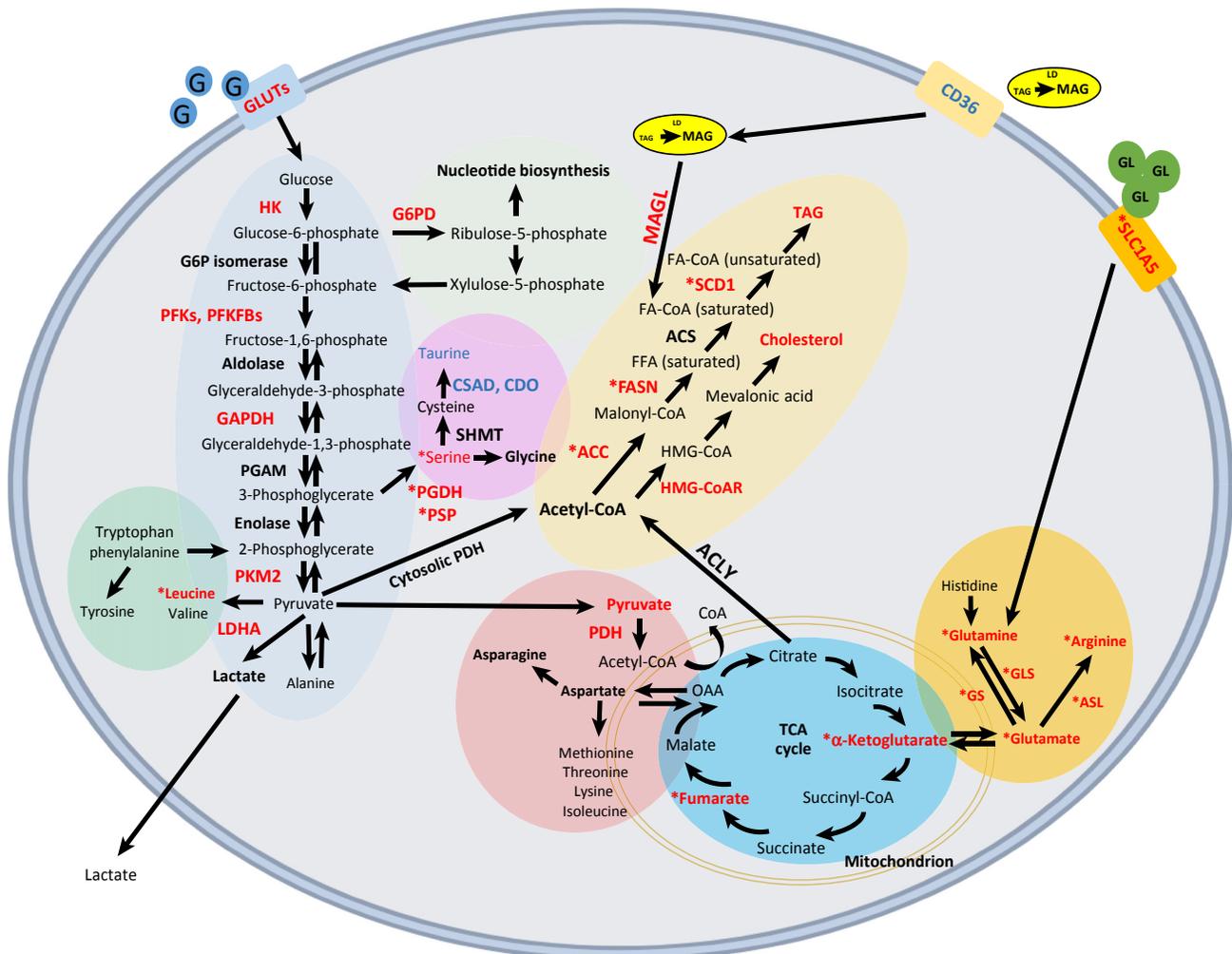
Mitochondrial respiration rates are higher in endocrine-resistant cells than in the parental cells. In tamoxifen-responsive cells, 4-hydroxy-tamoxifen (4-OHT) treatment reduces mitochondrial activity [104]. Elevated ER $\alpha$  expression increases the expression of NRF-1 and TFAM, leading to increased OXPHOS activity in endocrine-resistant tumors [105,106]. In addition, tamoxifen-stimulated mitochondrial ER $\beta$  has an antagonist role in breast cancer cells by increasing the concentrations of reactive oxygen species (ROS) in the mitochondria [107]. In addition, enhanced mitochondrial function and oxidative stress lead to tamoxifen resistance in MCF-7 breast cancer cells, and GCLC and NQO1 were suggested as potential biomarkers to target mitochondrial activation in tamoxifen resistance [108]. A recent biomarker study proposed more than 60 genes, including mitochondrial ribosomal and mitochondrial complex proteins, as novel mitochondrial biomarkers to predict early treatment failure and recurrence in patients treated with tamoxifen [109]. Horizontal transfer and packaging of mitochondrial DNA are significantly associated with the development of resistance to therapy. In addition, mitochondrial DNA acts as an oncogenic signal in cancer stem cells by sustaining OXPHOS-dependent endocrine therapy resistance [110]. Loss of one of the key tumor-suppressor genes, *RB1*, is associated with induction of mitochondrial protein translation and OXPHOS, leading to highly aggressive metastatic breast cancers with high OXPHOS activity [111].

Tumor-associated cells in the tumor microenvironment and the fuel sources used for mitochondrial respiration are crucial in endocrine resistance. Cancer-associated fibroblasts (CAFs) enable cancer cells to survive by providing lactate and ketone bodies to enhance their mitochondrial activity. Inhibition of mitochondrial activity with metformin and arsenic trioxide (ATO) overcomes fibroblast-induced tamoxifen resistance in ER $^{+}$  breast cancer cell lines [112]. In addition, increased PI3K/AKT pathway activity in CAFs facilitates multidrug resistance in both ER $^{+}$  and ER $^{-}$  breast tumors by exporting GPERs outside of the nucleus via the nuclear exporter CRM1 (XPO1). This mechanism was suggested to sustain excess pyruvate and lactate concentrations as a result of enhanced glycolysis and mitochondrial activity during the development of endocrine resistance [113]. Of note, CAFs also drive trastuzumab resistance in HER2 $^{+}$  breast cancers through expanding NF- $\kappa$ B, JAK/STAT, and PI3K/AKT pathways with increasing IL-6 expression [114].

Drug-resistant breast cancer cells have a distinct amino acid signature compared to non-resistant cells. The ratio between glutamine and glutamate levels is used as a biomarker for tumor aggressiveness and endocrine resistance in breast cancer cells [12,98]. Aromatase inhibitor-resistant breast cancer cell lines upregulate transporters involved in glutamine uptake [12,98]. In addition, tamoxifen-resistant cell lines are more sensitive to cysteine levels than are tamoxifen-sensitive lines [115]. Doxycycline-resistant MCF-7 breast cancer cells have higher levels of cysteine/methionine-regulating enzymes (such as CBS, MTHFR, and BHMT) than doxycycline-sensitive cells. These studies highlight the success of combination treatment strategies involving hormonal/targeted therapy agents in conjunction with agents targeting amino acid biosynthesis pathways.

Lipid metabolism is also altered during the development of drug resistance. Inhibition of ACC-1 via leptin and TGF- $\beta$  signaling causes elevation of acetyl-CoA, leading to recurrence and metastasis in breast cancer cells [116]. Furthermore, recent metabolic profiling data from several breast cancer studies revealed that nucleic acid and cholesterol synthesis pathways are activated during the development of tamoxifen resistance in breast cancer cells. Consistent

with these data, combining cholesterol-lowering medicines with endocrine treatment was shown to improve metabolic outcomes in endocrine-resistant breast cancer cells [117]. In addition, neutral lipids, lipid droplets, and free cholesterol accumulate in tamoxifen-resistant breast cancer cells compared to non-resistant cells [118]. Furthermore, differences in lipid metabolic pathways provide novel vulnerabilities in ER<sup>+</sup> tumors that might be targeted with available inhibitors [119].

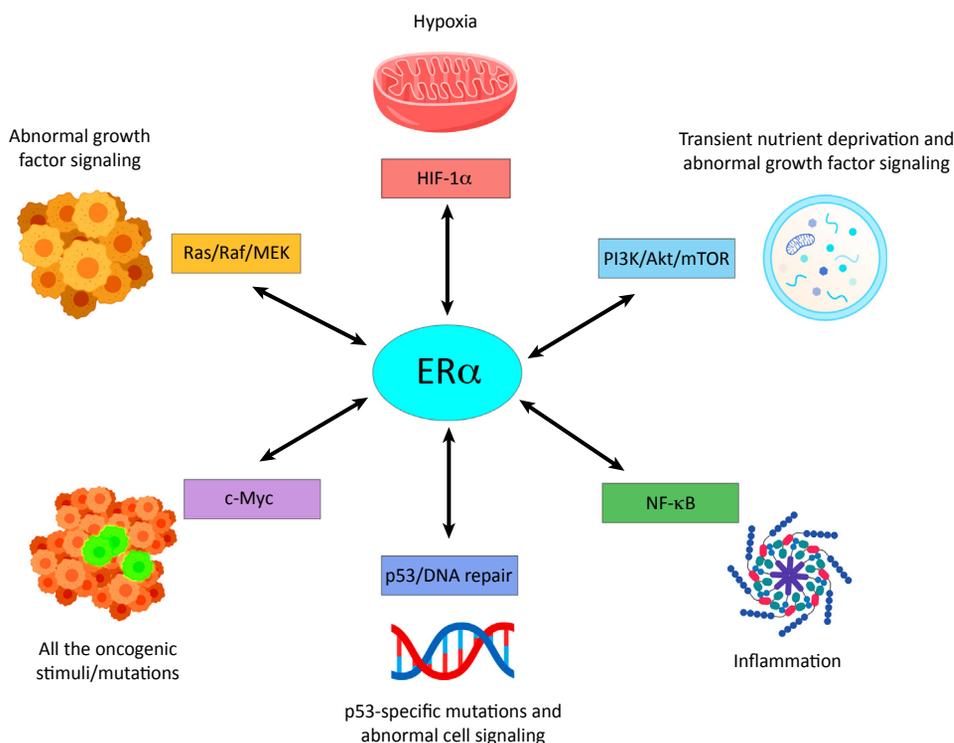


## Trends in Endocrinology &amp; Metabolism

**Figure 1. Metabolic Enzymes, Metabolites, and Macronutrient Transporters Regulated by Estrogen Signaling.** Enzymes and macronutrient transporters which are upregulated by estrogen signaling and high metabolite levels are shown in red. Enzymes and macronutrient transporters which are downregulated by estrogen signaling and low metabolite levels are shown in blue. \*Indicates high expression levels of metabolic enzymes, metabolites, and macronutrient transporters in breast cancers. Abbreviations: ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; ACS, acetyl-CoA synthase; ASL, argininosuccinate lyase; ATP S, ATP synthase; CD-36, cluster of differentiation 36; CDO, cysteine dioxygenase; CSAD, cysteine sulfinic acid decarboxylase; FASN, fatty acid synthase; FFA, free fatty acid; G, glucose; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GL, glutamine; GLUT, glucose transporter; G6P isomerase, glucose-6-phosphate isomerase; G6PD, glucose-6-phosphate dehydrogenase; GS, glutamine synthase; GSL, glutaminase; HK, hexokinase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMG-CoAR, 3-hydroxy-3-methylglutaryl-CoA reductase; LD, lipid droplet; LDHA, lactate dehydrogenase-A; MAG, monoacylglycerol; MAGL, monoacylglycerol lipase; OAA, oxaloacetate; PDH, pyruvate dehydrogenase; PFK, phosphofructokinase; PFKBP, phosphofructokinase 2/fructose biphosphatase; PGAM, phosphoglycerate mutase; PGDH, phosphoglycerate dehydrogenase; PKM2, pyruvate kinase isoenzyme M2; PSP, phosphoserine phosphatase; Q, quinone; SCD1, stearoyl-CoA desaturase-1; SLC1A5, solute carrier family 1 member 5; TAG, triacylglycerol; TCA cycle, tricarboxylic acid cycle.

### Liquid Biopsies To Detect the Metabolic Status of Tumors

The emerging field of liquid biopsies to diagnose patients, follow-up treatment responses, and monitor recurrence is gaining popularity in the clinic. The aim of the liquid biopsy is to collect blood, saliva, or urine samples from the patients to detect circulating tumor cells (CTCs) and/or other circulating factors (e.g., metabolites, DNA, or RNA). Following sample collection, metabolites in these samples can be analyzed with a wide array of techniques such as sequencing, nuclear magnetic resonance (NMR), and liquid or gas chromatography (LC/GC). This approach can be used for the early detection and genotyping of cancer cells [120], monitoring the metabolic effects of a treatment strategy, predicting drug response or resistance [121], metastatic capacity [122], or for detecting specific targetable mutations for therapy selection in breast cancers. For example, comparison of plasma samples from breast cancer patients and healthy individuals showed that high levels of cortisol, glutamine, L-arginine, linoleic acid, L-lysine, L-valine, uric acid, tyrosine, and phenylalanine were associated with breast cancer [123,124]. High levels of histidine, acetoacetate, glycerol, pyruvate, glycoproteins, mannose, glutamate, and phenylalanine were reported to be associated with metastatic phenotype in breast cancers [122]. Recently, several clinical trials used the identification of *PI3KCA* mutations in circulating tumor material to predict outcomes of inhibitor treatments targeting the PI3K pathway [125].



#### Trends in Endocrinology & Metabolism

**Figure 2. Abnormal Estrogen Signaling Stimulates Cell Proliferation and Growth in the Mammary Tissue.** This situation triggers various stress stimuli in a solid tumor, such as poor perfusion, transient nutrient deprivation and increased acidity. All these external factors activate different stress-associated pathways in the cell and eventually promote cell proliferation and create hypoxia in the core of the tumor. In addition, estrogen receptor (ER)-dependent regulator mechanisms are not unidirectional. These pathways also regulate genomic, extranuclear, and post-translational regulation of ERα, and upregulate several downstream targets to promote pathways associated with various hallmarks of cancer, such as angiogenesis, metabolic deregulation, drug resistance, and metastasis.

### Concluding Remarks and Future Perspectives

Estrogen signaling has a significant impact on how breast cancer cells rewire their metabolic pathways to meet their high energy demand during proliferation (Figures 1 and 2). These alterations can be seen in all aspects of macronutrient metabolism, including pathways associated with glucose, glutamine, and fatty acid metabolism. Early differences in metabolic phenotypes might inform clinicians about recurrence and have potential for use as biomarkers in the clinic to aid in therapy decisions for breast cancer. Liquid biopsy for circulating markers of metabolic phenotype of tumors offers early detection, monitoring, and prediction of therapy responses in clinic. Understanding how ERs regulate these pathways is crucial because this new knowledge will reveal new vulnerabilities to overcome drug resistance in different breast cancer subtypes. Specifically, further studies will be necessary to elucidate the crosstalk between estrogen signaling elements and key metabolic regulators to better target ER<sup>+</sup> breast cancers by combining endocrine therapy options with metabolic inhibitors (see Outstanding Questions).

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### Outstanding Questions

What are the main metabolic characteristics of endocrine-resistant tumors?

Are there metabolic phenotype differences between endocrine resistant-tumors that arise through different mechanisms?

What effects does genomic and non-genomic estrogen signaling have on glycolytic and mitochondrial oxidation pathways?

Does estrogen signaling directly regulate amino acid metabolism?

How do breast cancer cells rewire their metabolism during the development of endocrine resistance?

How do alterations in the tumor micro-environment and fuel sources contribute to the development of endocrine resistance?

Can we use metabolic changes as a biomarker of endocrine resistance in the clinic?

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