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Review

# Metabolic crosstalk in the breast cancer microenvironment

Ana S. Dias [a,b], Catarina R. Almeida [b], Luisa A. Helguero [b], Iola F. Duarte [a,*,1]

[a] *CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus de Santiago, Aveiro, Portugal*
[b] *iBiMED – Institute of Biomedicine, Department of Medical Sciences, University of Aveiro, Campus de Santiago, Aveiro, Portugal*

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**Abstract** During tumorigenesis, breast tumour cells undergo metabolic reprogramming, which generally includes enhanced glycolysis, tricarboxylic acid cycle activity, glutaminolysis and fatty acid biosynthesis. However, the extension and functional importance of these metabolic alterations may diverge not only according to breast cancer subtypes, but also depending on the interaction of cancer cells with the complex surrounding microenvironment. This microenvironment comprises a variety of non-cancerous cells, such as immune cells (e.g. macrophages, lymphocytes, natural killer cells), fibroblasts, adipocytes and endothelial cells, together with extracellular matrix components and soluble factors, which influence cancer progression and are predictive of clinical outcome. The continuous interaction between cancer and stromal cells results in metabolic competition and symbiosis, with oncogenic-driven metabolic reprogramming of cancer cells shaping the metabolism of neighbouring cells and vice versa. This review addresses current knowledge on this metabolic crosstalk within the breast tumour microenvironment (TME). Improved understanding of how metabolism in the TME modulates cancer development and evasion of tumour-suppressive mechanisms may provide clues for novel anticancer therapeutics directed to metabolic targets.


* *Corresponding author*: CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193, Aveiro, Portugal.
*E-mail address:* ioladuarte@ua.pt (I.F. Duarte).
[1] http://www.ciceco.ua.pt/ioladuarte

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## 1. Introduction

According to the Global Cancer Observatory, breast cancer (BC) was the cancer with highest incidence in 2018, with approximately 2 million cases registered worldwide, a number estimated to increase to over 3 million by 2040 [1]. Furthermore, BC is the leading cause of cancer-related deaths in women and the second-most lethal when considering both sexes. This high mortality is mostly related to advanced, metastatic cancer, as 5-year survival rates decrease from 99% in localized disease to 27% when the cancer has spread to distant body sites [2]. BC comprises several different tumour subtypes displaying high heterogeneity at the cellular and molecular levels. A commonly used classification criterion is based on the presence or absence of hormone receptors and epidermal growth factor receptor 2 (HER2) status, through which three groups are defined: oestrogen and/or progesterone receptor (ER/PR)-positive, HER2-positive and triple-negative breast cancer (TNBC; tumours that are both ER/PR- and HER2-negative). The presence of these receptors determines whether hormonal therapy and/or immunotherapy should be used. At the molecular level, BC subtypes are divided according to their gene expression signatures into luminal A, luminal B, HER2-enriched and the TNBC subtypes basal-like, claudin-low and metaplastic BC (Fig. 1). The hormone receptor−positive luminal A subtype presents the highest incidence and best prognosis, whereas TNBC subtypes are associated with the worst prognosis [3,4].

BC development, metastatic ability and response to therapy are determined not only by the characteristics of cancer cells but also by their interaction with the surrounding environment − the stroma. The tumour microenvironment (TME) is a dynamic entity composed of cancerous and non-cancerous cells, including fibroblasts, adipocytes, endothelial cells and immune cells (e.g. macrophages, lymphocytes, natural killer [NK] cells), soluble factors and extracellular matrix (ECM) components, which greatly influence malignant progression. Indeed, the stromal gene expression signature has been shown to be predictive of clinical outcome [5] and chemotherapy resistance [6] in BC. Moreover, changes in ECM proteins such as laminin content or expression of their receptors by cancer cells have been shown to determine BC cell growth and response to therapy [7–9]. These findings underline the importance of the microenvironment in the prognosis and treatment of BC and call for an improved understanding of cellular interactions in the TME.

Reprogramming of energy metabolism is recognized as one of the cancer hallmarks that actively contributes to cancer development [10,11]. Oncogenic events drive the dysregulation of metabolic pathways, which in turn provide cancer cells with selective advantages that enable their high proliferation and survival in a harsh microenvironment. Moreover, a rewired metabolism actively contributes to tumorigenesis via production of oncometabolites (e.g. 2-hydroxyglutarate), interaction with signalling pathways and metabolite-dependent epigenetic regulation. The altered metabolic program of cancer cells further impacts other cells residing in the TME and contributes to regulate processes deeply involved in cancer development, such as angiogenesis, inflammation and cancer immunity [12]. Hence, characterizing the metabolic interplay between cancer and non-cancerous cells in the TME may reveal key vulnerabilities of cancer and open new diagnostic and/or therapeutic perspectives.

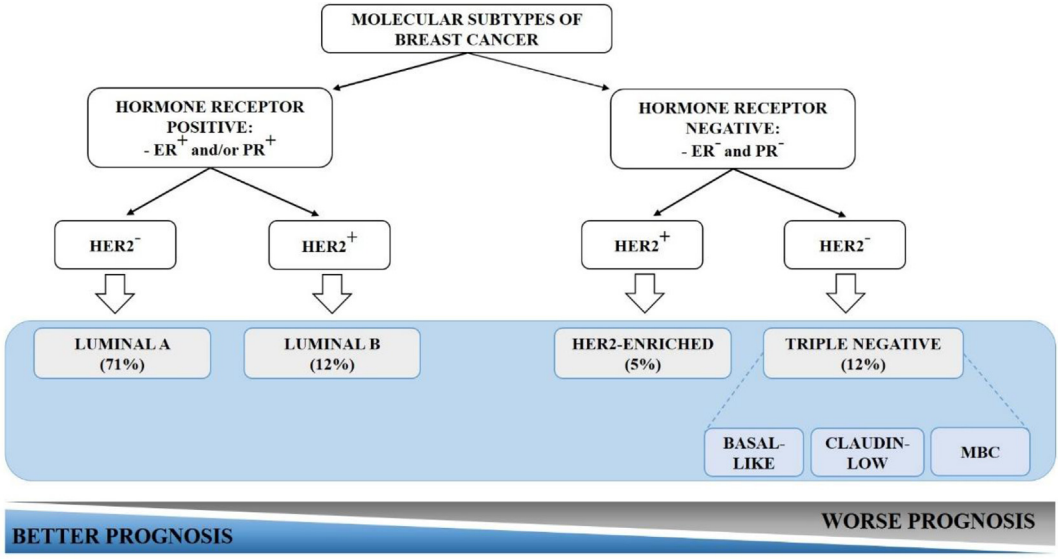


Fig. 1. Molecular subtypes of breast cancer (BC): luminal A, luminal B, HER2-enriched and triple-negative breast cancer (TNBC), comprising basal, claudin-low, normal-like and metaplastic breast cancer (MBC), and respective incidence, according to data in Ref. [2].

This review focuses on the metabolic interactions within the breast TME and their importance to BC biology and progression. A first section will be devoted to briefly characterising the breast TME, with emphasis on the known associations between the presence of specific non-cancerous cell types in the tumour niche and disease prognosis or clinical outcome. A summary list of studies addressing those correlations is provided in Table 1. Then, following an overview of the main metabolic dysregulations in BC tissues and cells, current knowledge on the metabolic crosstalk between BC cells and other cell types in the breast TME will be presented and discussed. Overall, this review offers an improved understanding of how metabolism modulates BC progression, and provides a solid background to aid the rational design of metabolic drugs to target the breast TME.

## 2. The breast tumour microenvironment

### 2.1. Immune cells in the breast tumour microenvironment

Cancer immunoediting is a dynamic process involving the interaction between neoplastic and immune cells, which greatly determines tumour progression and is currently divided into three phases: elimination, equilibrium and escape [13]. In the elimination stage, the most immunogenic cancer cells are recognized and eliminated by immune cells, such as macrophages, NK cells and $CD8^+$ T cells, mostly through cytotoxic mechanisms. At the equilibrium stage, which may last for several years, the tumour is kept in a dormant state, where tumour growth and metastasis are under immune control. However, some neoplastic cells eventually acquire insensitivity to immunological detection and/or elimination, evading the host immune system. Through this escape stage, the most aggressive clones adopt different strategies, such as recruitment of immunosuppressive cells and promotion of immune tolerance, thus contributing to an immunosuppressive TME and establishment of the malignant neoplasia [13,14].

The immune infiltrate in the TME is composed of cells from both lymphoid and myeloid lineages, which belong to the innate and adaptive immune systems, and vary considerably across different cancers and cancer subtypes [15–17]. As detailed in the following, although various immune cell subsets have been identified in the breast TME and found to be either anti- or pro-tumorigenic, the prevalence of cell populations traditionally classified as immunosuppressive or regulatory has often been correlated with BC aggressiveness and poor prognosis.

Tumour-associated macrophages (TAM) differentiate from circulating monocytes and are one of the immune cell types more abundantly present in the TME. These cells display great plasticity and polarize into different phenotypes according to microenvironment stimuli, such as oxygen availability, inflammatory mediators and other soluble factors. Higher numbers of TAM in the TME have been correlated with more aggressive disease and unfavourable prognosis in several cancer types, including BC [18,19]. Broadly, M2-like TAM can arise in response to anti-inflammatory and regulatory cytokines produced by Th2 cells, such as transforming growth factor beta-1 (TGF-β1) and interleukins IL-4, IL-10 and IL-13, and show pro-tumorigenic characteristics. On the other hand, M1-

Table 1
Stromal cells present in the breast TME and correlation with clinical outcome.

| Cell type | BC subtype | Expected outcome/main observations | Ref. |
|---|---|---|---|
| M2-like TAM | Basal-like | Poor outcome; Presence in more aggressive BC | [21–24] |
| TAN | n.d. | Immunosuppressive | [25,28] |
| pDC | n.d. | Poor outcome | [30,31] |
| TIL | $HER^+$ and TNBC | Favourable outcome; Correlation with tumour stage | [39] |
| CTLs | $HER^+$ and TNBC | Favourable outcome; Correlation with tumour stage; Better response to neoadjuvant chemotherapy | [35–38] |
| Th1 | n.d. | Favourable outcome | [40] |
| Th2 | Luminal | Poor outcome; Promotion of metastasis | [41] |
| Th17 | n.d. | Favourable outcome | [42] |
| Treg | n.d. | Poor outcome | [43–45] |
| NK cells | n.d. | Favourable outcome | [49] |
| MDSC | n.d. | Correlation with disease burden and clinical stage; Worse response to neoadjuvant chemotherapy | [56,57] |
| CAF | n.d. | Poor outcome; Promotion of tumour growth and invasion | [60] |
| Adipocytes | n.d. | Promotion of cancer cell proliferation; Relation with the development of chemotherapy resistance | [65,68,69] |
| Endothelial cells | TNBC | Relation with angiogenic response; Induction of invasiveness | [70,72] |

BC, breast cancer; CAF, cancer-associated fibroblasts; CTLs, lymphocytes; HER, human epidermal growth factor receptor; MDSC, myeloid-derived suppressor cells; NK, natural killer; pDC, plasmacytoid dendritic cells; TAM, tumour-associated macrophages; TAN, tumour-associated neutrophils; TIL, tumour-infiltrating lymphocytes; TME, tumour microenvironment; TNBC, triple-negative breast cancer; Treg, regulatory T lymphocytes; n.d. not defined.

like TAM occur on exposure to pro-inflammatory cytokines produced by Th1 cells, such as interferon (IFN)-γ and possess anti-tumoral functions. Other macrophage subtypes have been identified, some associated with tumours, which are not fully characterized yet [20]. In the breast TME, a higher infiltration of $CD163^+$, M2-like TAM has been correlated with unfavourable clinical features, such as larger tumour size, higher histological grade and ER negativity, as well as with higher tumour recurrence and lower patient survival [21–24].

Circulating neutrophils are also recruited by chemotractants to the TME, where they become tumour-associated neutrophils (TAN). Like macrophages, these cells can be polarized into either pro- or anti-tumorigenic phenotypes, which influence tumour development in different ways [25]. TAN infiltration has been correlated with poor outcomes in several cancer types [26], although conflicting data exist in this respect [27]. In BC, the only study so far showed that TAN were present in nearly 90% of the analysed TNBC tissues, whereas more moderate percentages were found for HER2-positive and hormone receptor–positive/HER-negative tumours (53% and 5%, respectively). However, as the phenotypic and functional features of infiltrating TAN were not assessed, their role in BC progression remains unclear.

Dendritic cells (DC) are professional antigen-presenting cells that play a central role in cellular immune responses against cancer [29]. Both immature and mature dendritic cells of myeloid origin (mDC) have been found to frequently infiltrate early-stage breast carcinomas, but only mature mDC infiltration correlated with axillary lymph node involvement and the Scarff-Bloom-Richardson grading index, used as an indicator of tumour aggressiveness [30]. Furthermore, infiltration by plasmacytoid dendritic cells (pDC), although seldom observed, was strongly associated with decreased overall survival and relapse-free survival [30]. Accordingly, higher levels of circulating pDC, likely reflecting lower infiltration of the tumour site, were shown to be a good prognostic factor for BC [31]. The negative impact of pDC infiltration has been attributed to the impaired capacity of tumour-associated pDC to produce type I interferon, which could result in higher proliferation of pro-tumorigenic regulatory T lymphocytes (Treg) [32]. Recently, the phenotype of DC subsets in BC has been further scrutinized, based on the transcriptomic profiling of tumour and adjacent noninvolved tissues [33]. In TNBC, all DC subsets showed enrichment for the interferon pathway, whereas in luminal BC, the transcriptional signatures were specific to each DC subset, with pDC displaying enrichment for the vascular wound healing and extracellular matrix pathways.

Tumour-infiltrating lymphocytes (TIL) comprise B and T cells, with cytotoxic $CD8^+$ T lymphocyte (CTL) cells being the most abundant TIL in the BC microenvironment, followed by helper $CD4^+$ T cells and NK cells [34]. The association between the abundance of CTLs and good prognosis of primary breast tumours and/or pathological complete response to neoadjuvant chemotherapy (NAC) has long been recognised and confirmed by recent studies involving large cohorts [35–38]. In addition, lower lymphocytic infiltration was found in tumours at first metastatic recurrence compared with primary ones, which suggests that immune escape is important during metastatic progression [39]. As for $CD4^+$ T helper cells, their role in BC is less clear. Pro-inflammatory Th1 cells, which typically produce IFN-γ, have been correlated with improved survival in patients with BC [40]. By contrast, anti-inflammatory Th2 cells, which produce IL-4, have been shown to promote metastasis in luminal BC [41]. Other important subsets of $CD4^+$ T cells are Th17 cells and regulatory T cells (Treg or $FOXP3^+$ T cells). A significantly higher number of Th17 effector cells have been reported in breast tumours compared with normal breast tissue [42]. These cells appear to be involved in anti-tumour responses and are associated with a more favourable prognosis. On the other hand, an inverse correlation was found between the frequency of Treg in BC and patient survival [43–45]. Treg are suppressor cells required for self-tolerance, and their balance in relation to Th17 cells is considered crucial in the regulation of cancer progression [46]. Furthermore, the frequency of circulating Treg was found to be dependent on the BC subtype, with HER2-positive BC showing significantly higher Treg frequencies than HER2-negative patients at the same clinical stage [47].

NK cells are innate lymphocytes that play an important role in tumour immune surveillance, inhibition of growth and prevention of metastatic dissemination [48]. Increased NK cell infiltration has been associated with improved prognosis of several solid tumours, including BC [49]. In patients with large and locally advanced BC, the abundance of NK cells in the peritumoral space has been correlated with a good pathological complete response to NAC [49]. Moreover, human BC samples resistant to NAC displayed lower NK cell infiltration than the tumours sensitive to treatment, suggesting that NK counting could be of value in predicting response to treatment [50]. It has also been shown that BC of luminal, HER2-positive and basal-like subtypes differentially express various NK cell–activating ligands. Natural killer, group 2, member D (NKG2D) ligands and the DNAX accessory molecule-1 (DNAM-1) ligands were reported as the most common, particularly in luminal tumours that express low levels of the inhibitory human leukocyte antigen (HLA) transcripts [51]. Poorly immunogenic cancer cells frequently loose expression of classical major histocompatibility complex class I molecules (HLA-A, HLA-B and HLA-C) to escape from CTL attack, while they become more vulnerable to NK cell-

mediated anti-tumour immunity [52]. In breast tumours with low expression of these molecules, increased expression of HLA-G and HLA-E was correlated with higher relapse risk, which suggests that these non-classical HLA molecules could have a role in counteracting the susceptibility to attack by NK cells [53].

Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of immature myeloid cells which inhibit immune responses through several processes, including depletion of arginine (required for T cells survival), production of reactive nitrogen species and reactive oxygen species (ROS), and secretion of inhibitory cytokines [54]. The levels of MDSC in blood have been found significantly increased in patients with BC relative to healthy controls [55], as well as in patients with higher disease burden and clinical stage, suggesting that MDSC levels could be predictive of patient survival [56]. In addition, patients with BC with reduced circulating levels of MSDC were found to respond better to NAC, whereas poor responders showed increased MSDC levels, which further increased on treatment [57]. Recently, Gonda *et al.* provided additional evidence for the prognostic value of MDSC quantification in peripheral blood of patients with BC [58]. Among preoperative patients, the overall survival was significantly shorter for those where MDSC represented more than 1% of peripheral blood mononuclear cells. Comparing all study subjects, circulating MDSC levels were significantly elevated in preoperative and recurrent patients with BC compared with postoperative patients, chemotherapy-treated patients or healthy controls. The role of MDSC in immune suppression was postulated to involve multiple pathways, such as increased production of IL-6, suggesting a Th-2 dominant status, and decreased production of IL-12 by dendritic cells. Furthermore, MDSC levels also correlated with protein turnover and C-reactive protein levels, which reflects a relationship with malnutrition and inflammation, respectively [58].

### 2.2. Non-immune cells in the breast tumour microenvironment

Non-immune cells, including cancer-associated fibroblasts (CAF), adipocytes and endothelial cells, are also in close interaction with proliferating tumour cells and constitute an integral part of the TME, playing a multifaceted role in tumour initiation and progression [59].

CAF are a specialized group of fibroblasts characterized by irreversible activation and evasion of apoptosis [60]. CAF may be derived from various cells, namely resident fibroblasts, epithelial, endothelial and mesenchymal cells, and constitute a heterogeneous and abundant population in the TME. CAF are believed to promote the growth and invasion of cancer cells through multiple mechanisms, including the secretion of growth factors and pro-inflammatory mediators, cell–cell interactions and metabolic crosstalk with tumour cells [60]. Incubation of BC cell lines with CAF isolated from human invasive breast carcinomas produced an upregulation of several oncogenes in BC cells and increased their invasion ability [61]. In addition, CAF cocultured with endothelial cells (HUVEC) were shown to promote angiogenesis [61]. In the clinical setting, significant correlations between CAF infiltration and worse BC clinical outcome were reported based on a meta-analysis [62]. Specifically, high density of activated fibroblasts in primary BC correlated with decreased patient survival, poor tumour differentiation and lymph node metastasis, thereby highlighting the prognostic value of CAF infiltration in BC. Recently, Costa et al shed some light into the mechanisms through which BC CAF contribute to an immunosuppressive microenvironment [63]. Four CAF subsets (S1 to S4) were identified and found to be differentially distributed in human normal breast tissues and BC subtypes. Then, the CAF-S1 subset, particularly enriched in TNBC tissues, was highlighted as a major contributor to immunosuppression. This was explained by their ability to recruit CD4+CD25 + T cells and to promote their differentiation into Treg cells.

Mature adipocytes play an active role in BC progression, as supported by the well-established epidemiological link between obesity and postmenopausal BC risk [64]. As recently reviewed [65], multiple factors are involved in the pro-tumorigenic functions of adipocytes, such as production of adipokines (e.g. leptin, adiponectin) and other soluble factors (e.g. IL6, TNF-α), extracellular matrix remodelling, transformation into CAF and metabolic rewiring. On tumour invasion, with the disruption of the basement membrane, BC cells and adipocytes are in close contact, which leads to several phenotypic and functional alterations in both cell types. Adipocytes surrounding the invasive tumour front were found to exhibit, both *in vitro* and *in vivo*, reduced lipid content and expression of adipocyte markers, together with an activated state characterized by overexpression of proteases and pro-inflammatory cytokines [66,67]. On the other hand, several molecular changes were observed in TNBC cells cocultured with adipocytes, such as the induction of pro-inflammatory genes and increased migratory capacity [68]. Furthermore, adipocytes have been implicated in the development of chemotherapy resistance in several cancer types, including BC [65]. A recent study has shown that adipocytes (differentiated *in vitro* and isolated from lean and obese patients) could directly regulate the response of BC cells to doxorubicin and other drugs, independently of BC subtype [69]. The upregulation of the transport-associated major vault protein (MVP) by adipocytes was found to mediate reduced drug accumulation in the nuclei of BC cells and drug efflux from cytoplasmic vesicles to the extracellular medium, thus inducing a multidrug-resistant phenotype.

In human BC tissues, higher MVP expression was found at the tumour invasive front, where cancer cells are closer to adipocytes, suggesting that MVP-mediated drug resistance may be clinically relevant regarding the formation of chemoresistant metastasis.

Endothelial cells (EC) in the lining of blood vessels are also important players in the breast TME, particularly regarding the angiogenic support of tumour growth and metastatic spreading. Indeed, the reciprocal growth factor exchange between cocultured endothelial and BC cells has been shown to directly modulate the angiogenic response and tumour invasion capacity [70]. Direct cell-to-cell contact is required for endothelial cells to support the *in vitro* survival, stemness and invasiveness of BC cells under starvation [71]. A particularly important observation was that EC could enhance mammary cells stemness *in vitro* and their tumour initiation potency *in vivo*, which highlights their role in providing a pro-tumorigenic niche in a perfusion-independent manner. The influence of EC over the metastatic potential of TNBC cells has also been recently addressed [72]. A model has been proposed where TNBC, treated with TGF-β to induce endothelial–mesenchymal transition, increase the secretion of plasminogen activator inhibitor 1 (PAI-1), which in turn augments the secretion of CCL5 by cocultured EC, followed by its binding to TNBC membrane. This results in induction of TNBC invasiveness and further stimulates PAI-1 production, creating a positive feedback loop. In addition, in tissues of patients with TNBC, PAI-1 expression was correlated with angiogenesis and distant metastasis, underscoring the potential clinical relevance of targeting PAI-1 to hamper TNBC metastization.

## 3. Breast cancer metabolic reprogramming

Several studies have identified extensive differences between the metabolic profiles of BC and normal breast tissues, suggesting alterations in various metabolic pathways such as glycolysis, the TCA cycle, amino acid, nucleotide and/or lipid metabolisms [73–75]. In addition, BC tissue metabolic composition has been reported to depend on multiple factors such as tumour size and grade [76], race and age [74], and hormonal status [77,78], which reflects BC heterogeneity and complexity. Interestingly, in a study gathering metabolite, protein and gene expression data from over 200 breast tumours, three different metabolic clusters could be identified which did not correlate with ER status, contributing with additional information to understand BC heterogeneity [79]. In particular, these clusters were found to differ mainly in glycolysis/gluconeogenesis and in glycerophospholipid metabolism. Metabolic profiling of cultured BC cells also revealed high metabolic variability among different cell lines [80,81] and helped to corroborate many of the features found in tissues. For instance, ER-positive cells (BT-474) were found to be less glycolytic than ER-negative cells (MDA-MB-231 and MDA-MB-453) [82], similarly to the observation made in tissues [78]. On the other hand, metabolic changes in TNBC cells indicated increased energy demand, consistent with the more aggressive clinical behaviour of this cancer subtype [82,83]. In the following sections, current knowledge on the functional importance and regulation of specific metabolic alterations found in BC tissues and cells will be addressed. Fig. 2 provides an overview of the main features of BC metabolic reprogramming.

### 3.1. Glycolysis

Glycolysis entails a series of reactions where glucose is converted to pyruvate, with the concomitant production of NADH and ATP. The resulting pyruvate can either be converted to acetyl-CoA and enter the TCA cycle or give rise to lactate, especially if oxygen availability is low. Elevated glucose uptake and glycolysis, together with high lactate production even under aerobic conditions, are a recognized hallmark of many cancers [84]. This so-called Warburg effect is thought to confer selective advantages to cancer cells, such as rapid energy production, substrates for biosynthetic pathways, redox equilibrium and promotion of tumour invasion [84]. Importantly, enhanced glucose uptake and glycolytic activity are at the basis of the noninvasive imaging technique $^{18}$F-fluorodeoxyglucose positron emission tomography, which is widely used in the clinic as a diagnostic and therapy response monitoring tool [85]. In BC, enhanced glycolysis has been observed in human neoplastic tissues compared with non-cancerous ones [73,86], as well as in tumour cell lines, compared with normal breast epithelial cells [82,87]. Several factors have been shown to contribute to enhanced glycolytic activity in BC.

In human cells, glucose uptake is facilitated by a family of membrane glucose transporter proteins designated as GLUTs. Among the 14 isoforms encoded by the human genome, GLUT1 is most widely distributed in body tissues and often overexpressed in cancer, including BC [88]. Using immunohistochemical analysis of tissue microarrays, Choi et al have shown GLUT1 to be overexpressed in TNBC, especially in the basal-like subtype, and to correlate with a more glycolytic profile [89]. In another study, GLUT1 and GLUT3 were reported to be significantly more expressed (both at mRNA and protein levels) in poorly differentiated tumours of grades 2 and 3 compared with grade 1 well-differentiated tumours [90]. Moreover, in a recent meta-analysis, tissue overexpression of GLUT1 was found to significantly correlate with worse prognosis and poor survival in BC [88].

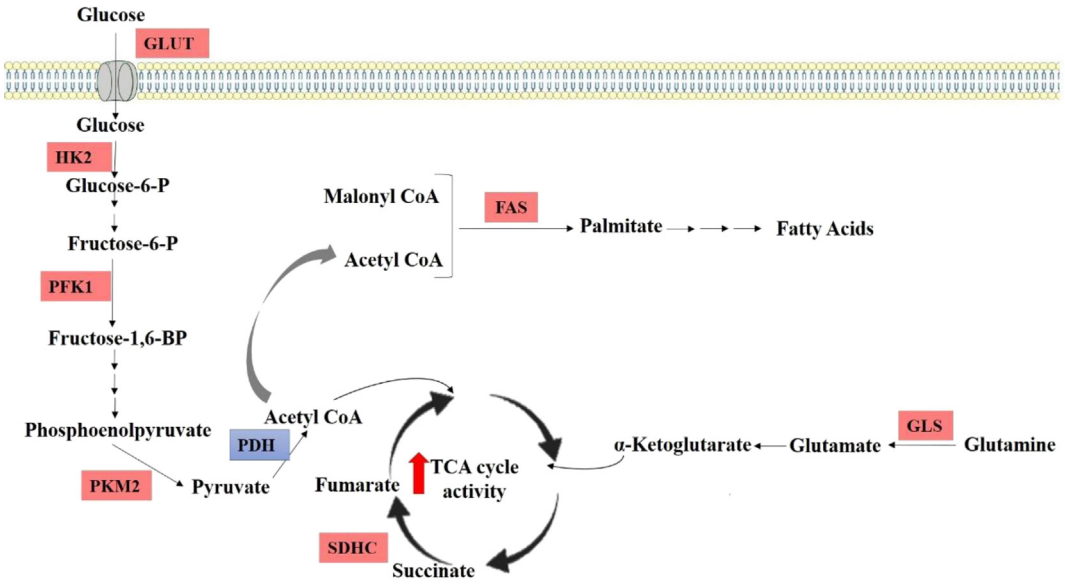


Fig. 2. Overview of metabolic reprogramming in breast cancer cells. Main changes in glucose metabolism comprise overexpression of glucose transporters (GLUT) and the glycolytic enzymes hexokinase 2 (HK2), phosphofructokinase 1 (PFK1) and pyruvate kinase M2 (PKM2); TCA cycle alterations include decreased expression of the enzyme pyruvate dehydrogenase (PDH) and overexpression of the succinate dehydrogenase complex (SDHC); other metabolic shifts in relation to normal cell metabolism comprise increased glutamine consumption, together with increased expression of glutaminase (GLS), increased lipid synthesis and expression/activity of fatty acid synthase (FAS). The proteins overexpressed/decreased in BC are highlighted in red/blue, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The first step of glycolysis, whereby glucose is converted to glucose-6-phosphate, is catalysed by hexokinases (HK). Among the 4 isoforms found in mammalian tissues, HK2 is reported to be particularly overexpressed in cancer tissues [91]. HK2 was found to be expressed in nearly 80% of 27 untreated primary BC analysed by immunohistochemistry [92], corroborating earlier findings [93]. More recently, HK2 was shown to be required for tumorigenesis in mouse models of ErbB2/Her2-driven BC, while HK2 ablation inhibited the malignant phenotype of BC cells both *in vitro* and *in vivo* [94].

The activity of phosphofructokinase (PFK1), another rate-limiting enzyme of glycolysis which converts fructose-6-phosphate to 1,6-bisphosphate, has also been found increased in BC tissue compared with either normal breast tissue [95] or to adjacent paracancer tissue [96]. Moreover, increased protein expression of total PFK-1 accompanied elevated enzyme activity, while different isoenzyme patterns were found to characterize cancer and paracancer breast tissues [96].

The third committed step of glycolysis, whereby phosphoenolpyruvate (PEP) is transformed into pyruvate, is catalysed by pyruvate kinases (PK). The isoform PKM2 is highly expressed in highly proliferating cells in several cancer types. Besides having a key role in glycolysis, PKM2 contributes to tumorigenesis by acting as a coactivator and protein kinase [97]. In BC, the clinical significance of elevated PKM2 has been recently addressed in a meta-analysis, whereby high PKM2 expression was indicative of worse survival in patients with BC and correlated with lymph node metastasis [98].

The regulatory role of different genes and transcription factors over glycolytic activity in BC has also been investigated in a few studies. The BC susceptibility gene 1 (BRCA1) is a major tumour suppressor gene and the most frequently mutated gene in hereditary BC [99]. In BC cells, BRCA1 was shown to downregulate glycolysis and to upregulate the TCA cycle coupled to oxidative phosphorylation [100]. Inactivation of the phosphorylated form of RAC-alpha serine/threonine-protein kinase (AKT) by BRCA1 was postulated to account for this reversion of the Warburg effect. Additionally, Zhao and co-workers published the first evidence associating overexpression of the ERBB2/HER2 oncogene with increased glycolysis. BC cell lines (MCF7 and MDA-MB-231) overexpressing ERBB2 displayed enhanced glucose uptake, lactate production and decreased oxygen consumption, through the upregulation of heat shock factor 1 (HSF1) [101]. Hypoxia-inducible factors (HIF) 1 and 2 are important mediators of oxygen level regulation, which is especially relevant in cancer, given that almost all solid tumours have poorly oxygenated areas. In hypoxic conditions, both HIF-α subunits (HIF-1α and HIF-2 α) escape proteolysis and dimerize, with the HIF-1β subunit activating the transcription of target genes [102]. Different roles for HIF-1α and HIF-2α have been found in human BC cell lines [103,104]. Activation of HIF-1α has been reported to promote a shift towards aerobic glycolysis, increased glucose

uptake and lactate release in BC cells [103]. Corroborating these findings, the higher glycolytic activity of aggressive metastatic BC cells was found to be mostly driven by HIF-1α [104]. On the other hand, activation of HIF-2α was found to induce overexpression of proteins involved in tumour growth and cell proliferation, such as epidermal growth factor receptor (EGFR), RAS and cyclin D1 [103]. The transcription factor TWIST has also been implicated in the reprogramming of glucose metabolism in BC cells, as it was found to promote overexpression of several glycolytic genes, by inducing β1-integrin/FAK/AKT/mTOR signalling and inhibiting p53 signalling pathways [105].

### 3.2. TCA cycle and oxidative phosphorylation

The TCA cycle comprises a series of reactions occurring in the mitochondria, whereby acetyl-CoA is oxidized, resulting in carbon dioxide and ATP, together with the reduced cofactors NADH and FADH2. These cofactors will pass their electrons into the electron transport chain allowing for ATP generation through oxidative phosphorylation (OXPHOS). While quiescent cells rely mainly on oxidative mitochondrial metabolism for energy production, cancer cells typically display upregulated glycolysis and mitochondrial dysregulation [106]. Indeed, mutations in mitochondrial DNA, including those affecting respiratory complex I and OXPHOS, were detected in breast tumours and cell lines and postulated to regulate cancer metastasis [107,108]. Furthermore, different TCA cycle–related enzymes were found to be altered in BC. Pyruvate dehydrogenase complex (PDH) catalyses the oxidative decarboxylation of pyruvate into acetyl-CoA and therefore controls the flow of metabolites from glycolysis to the TCA cycle and the subsequent generation of ATP by mitochondrial metabolism. Recently, reduced expression of PDHX, a structural component of the PDH complex, was reported in human breast tumour tissues and correlated with low patient survival [109]. In addition, in BC cell lines, suppression of PDHX led to decreased mitochondrial oxidation, promotion of extracellular acidification and increased cell proliferation [109]. The succinate dehydrogenase (SDH) complex is a key enzyme of the TCA cycle (whereby the subunit SDHA catalyses the conversion of succinate to fumarate) and directly participates in cellular respiration as complex II of mitochondria respiratory chain. In human BC tissues, SDH expression was found to be dependent on the BC molecular subtype, with HER-2 and luminal A subtypes showing, respectively, higher and lower expression of the SDHA subunit [110,111]. In TNBC cells, transfection with BRCA1 induced increased SDHC gene expression, which resulted in lower succinate levels (thereby promoting HIF1α degradation and glycolysis downregulation), as well as in increased ATP production via enhanced OXPHOS [100].

### 3.3. Amino acid metabolism

Besides glucose, some amino acids such as glutamine and serine are important substrates for neoplastic cells to grow and proliferate. Several tumours are ‘addicted’ to glutamine, displaying a markedly increased consumption of this amino acid. Glutamine is required not only for biosynthetic pathways (amino acids and nucleic acids production), but also for glutaminolysis, whereby it is converted into TCA cycle intermediates and lactate, providing an extra energy source for cancer cells [112]. Once taken up by cancer cells, glutamine is converted into glutamate by glutaminase (GLS). This enzyme was found to be differentially expressed in human tumour tissues depending on the BC subtype [113]. Specifically, GLS expression was reported to be higher in TNBC than in luminal and/or HER2 subtypes. Accordingly, both isoforms of GLS, mitochondrial phosphate-activated glutaminase C and the kidney-type glutaminase, were recently reported to be essential for the survival and proliferation of TNBC cells, as well as to be required for the growth of TNBC xenografts [114]. Moreover, when comparing basal and luminal BC cell types, the latter was reported to be more glutamine-independent, due to lineage-specific expression of glutamine synthetase [115].

Serine is a non-essential amino acid whose biosynthesis is often upregulated in cancer cells [116]. The first step of the serine biosynthetic pathway is catalysed by the enzyme 3-phosphoglycerate dehydrogenase (PHGDH), which is genetically amplified and/or overexpressed at the protein level in several cancer types, including BC. Specifically, PHGDH protein levels were found elevated in a significant portion of ER-negative [117] and human breast tumours [118], as well as in several BC cell lines [119]. PHGDH overexpression has been associated with promotion of breast tumour growth via several mechanisms, including fuelling of protein synthesis and one-carbon metabolism (hence supporting nucleotide synthesis, antioxidant defence and methylation reactions) [120], promotion of TCA cycle anaplerosis [117] and overproduction of the oncometabolite D-2-hydoxyglutarate [121].

### 3.4. Lipid metabolism

Highly proliferative cells, such as cancer cells, have elevated demand for lipids and cholesterol. To satisfy their needs, cancer cells take up more exogenous lipids and lipoproteins and/or activate *de novo* lipogenesis and cholesterol biosynthesis [122]. Increased fatty acid (FA) synthesis and upregulation of membrane lipids were observed in BC tissues [123], which, together with decreased levels of free fatty acids (FFA) [73], suggests newly synthetized FA to be rapidly used in the production of membrane phospholipids. In addition, FAs may be used as energetic fuels and as substrates for the

synthesis of complex lipids, such as triacylglycerols (TAG), ceramides and inflammatory mediators such as prostaglandins. Interestingly, tumour subtype–dependent differences in lipogenesis and FA use were found in BC tissues [78] and cells [124]. In particular, a preferential incorporation of palmitate into storage TAG was reported in TNBC cells, whereas luminal cells preferentially shunted palmitate into FA oxidation [124].

The rate-limiting enzyme in *de novo* lipogenesis is fatty acid synthase (FAS), which catalyses the final steps of FA biosynthesis, whereby malonyl-CoA and acetyl-CoA act as substrates to generate palmitate, in the presence of NADPH. Importantly, *FASN*, the gene which encodes FAS, has been considered an oncogene in BC [125]. In both cancer tissues [126] and cells [127], FAS was found to be more abundantly expressed in the BC subtype HER2-positive and to have lower expression in TNBC. Moreover, HER2 was reported to complex with FAS, promoting its phosphorylation and, hence, increased enzymatic activity, thus boosting cancer cells proliferation [127].

Choline consumption by cancer cells has been associated with its increased utilization for anabolic reactions and high proliferation rates, a metabolic dependence not observed in rapidly proliferating untransformed cells [128]. Luminal BC cells [129] and TNBC cells [130] showed upregulation of choline metabolism, comprising high choline uptake and conversion to phosphocholine. In addition, phospholipase D (PLD), which catalyses the hydrolysis of phosphatidylcholine to phosphatidic acid and choline, has been found overexpressed in human breast tumours compared with normal breast tissues [131]. Interestingly, phosphatidic acid was proposed to enhance the metastatic capabilities of BC cells [132]. Moreover, different BC cell lines displayed variable PLD activity, which possibly correlated with the cells proliferative activity [132].

## 4. Metabolic interplay in the breast tumour microenvironment

The continuous crosstalk between malignant and stromal cells is an integral part of tumour pathophysiology, of which cell metabolism is an important facet [59]. This section focuses on the reciprocal metabolic interplay between BC cells and some types of stromal cells present in the TME, namely TAM, TIL, MDSC, CAF and adipocytes. Fig. 3 provides an overview of the main metabolic interactions reported. Even though other stromal cells such as endothelial cells, neutrophils and DC are present in BC TME, studies on their metabolic interplay with BC cells are missing and more research is needed to fulfil this gap.

### *4.1. Metabolic interplay between breast cancer cells*

Breast tumours typically comprise well-vascularized, oxygenated cells (negative for HIF-1α), which express monocarboxylate transporter 1 (MCT1), along with poorly vascularized and hypoxic BC cells (positive for HIF-1α), which express hypoxia-induced MCT4 [133,134]. Hypoxic cancer cells use MCT4 to release the abundantly produced glycolytic lactate, which in turn is

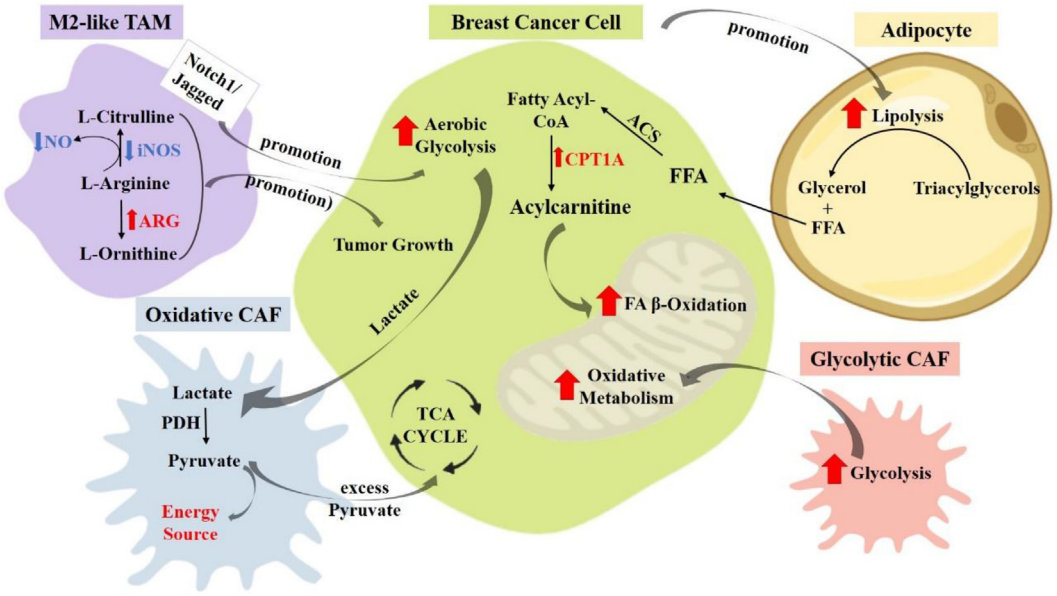


Fig. 3. Metabolic interplay between BC cells and different non-cancerous cells present in the TME, namely tumour-associated macrophages (TAM), cancer-associated fibroblasts (CAF) and adipocytes. ACS: acyl-CoA synthetase; ARG: arginase; CPT1A: carnitine palmitoyltransferase; FFA: free fatty acids; iNOS: inducible nitric oxide synthase; NO: nitric oxide; PDH: pyruvate dehydrogenase complex.

imported by oxygenated BC cells via MCT1. These cells take up lactate to fuel their mitochondrial metabolism, with alanine and glutamate being the predominant lactate-derived metabolites. As oxygenated cells take up lactate even in the presence of glucose, they request lower levels of glucose, which freely diffuses to fuel glycolysis-dependent hypoxic cells, supporting tumour cell survival under both normoxia and hypoxia [133,135]. Notably, targeting this lactate-based metabolic symbiosis through MCT4 ablation was shown to surmount resistance to anti-angiogenic therapy in a mouse model of BC [134]. Furthermore, lactate may act not only as a substrate but also as a signalling metabolite. In particular, lactate accumulated in hypoxic BC cells was shown to stabilize the NDRG3 protein, a PHD2-interacting protein negatively regulated by oxygen, thereby activating the Raf-ERK pathway to promote tumour growth and angiogenesis [136]. Glutamine is another metabolite considered to be involved in the metabolic symbiosis between BC cells. *In vitro* coculture experiments with glutamine-dependent (basal-type) and glutamine-independent (luminal-type) BC cells showed that the latter could rescue the first through the ability to synthetize glutamine, suggesting a possible glutamine symbiosis that would occur within the breast ducts between basal and luminal epithelial cells [115].

### 4.2. Metabolic interplay between tumour-associated macrophages and breast cancer cells

In general, anti-tumoral macrophages have been associated with increased glycolysis, whereas pro-tumoral macrophages have an intact TCA cycle leading to OXPHOS metabolism [137]. Stimulation of either murine or human monocytes/macrophages specifically with breast tumour extracts has been shown to induce a glycolytic phenotype in TAM-like macrophages, suggesting rewiring of glucose metabolism to be involved in TAM differentiation and functional features [138]. Specifically, by using a proteomics approach, the glycolytic enzyme HK2, as well as the downstream proteins ATP-dependent 6-phosphofructokinase (PFKL) and enolase 1 (ENO1) were found significantly upregulated in TAM-like cells stimulated with tumour extracts. On the other hand, compared with normoxic TAM, hypoxic TAM isolated from a murine BC model displayed reduced glycolysis mediated by mTOR inhibition because of overexpression of the regulated in development and DNA damage response 1 (REDD1) gene [139]. Such lower utilization of glucose by hypoxic TAM was linked to its increased use by endothelial cells and promotion of angiogenesis. Importantly, this could justify the limited success of mTOR inhibitors in anticancer therapy, as their antitumour effects could be counteracted by the pro-angiogenic response promoted by TAMs.

Incubation of human macrophages with lactate has also been shown to promote the TAM-like phenotype and to strongly stimulate the secretion of the CCL5 chemokine through Notch signalling activation [140]. In turn, CCL5 promoted glycolysis in BC cells via AMPK activation, and induced the epithelial–mesenchymal transition (a main mechanism underlying cancer metastasis). Hence, CCL5 and its receptor in BC cells (CCR5) were highlighted as important therapeutic targets. On the other hand, the G protein-coupled receptor 132 (GPR132) expressed by macrophages was reported to be crucially important in sensing and responding to the lactate released by cancer cells, and hence, in M2-like polarization [141]. The clinical relevance of these findings has been corroborated by showing that Gpr132 expression in BC tissues positively correlated with TAM infiltration, metastasis and poor prognosis.

Arginine metabolism has also been implicated in the crosstalk between BC cells and TAM. In particular, M2-like TAM differentiated from human monocytes cocultured with TNBC cells displayed significantly downregulated citrulline production [142]. L-citrulline and nitric oxide (NO) are generated from L-arginine in a reaction catalysed by inducible nitric oxide synthase (iNOS) and are both typically overexpressed in M1-like macrophages. On the other hand, M2-like macrophages use arginine as a substrate for arginase 1 (ARG1), which hydrolyses arginine to ornithine and urea [143]. Hence, decreased citrulline in TNBC-educated macrophages is in accordance with the pro-tumour functions of these cells. The capacity of TAM to produce nitric oxide (NO) has also been found reduced in an animal model of BC, due to decreased expression of iNOS. Notably, this effect was more pronounced in macrophages within the tumour, than in more distally located peritoneal-elicited macrophages [144].

### 4.3. Metabolic interplay between tumour-infiltrating lymphocytes and breast cancer cells

Cancer cells and CTLs share a number of metabolic features, which include upregulated glycolysis and intense anabolic activity to satisfy increased energetic and biosynthetic demands [145]. Several studies have established that the metabolic competition between these cells in the microenvironment significantly impacts tumour progression. For instance, tumour avidity for glucose, which becomes less available in the extracellular medium, has been shown to dampen glycolysis in T cells and to suppress their tumoricidal functions, namely in sarcoma [146] and melanoma [147] mouse models. Furthermore, abundant lactate production by tumour cells has been reported to hinder MCT1-mediated lactate export by TIL, required to sustain high glycolytic activity, which results in decreased proliferation, cytokine production and/or cytolytic capacity [148–150]. Although the previously cited studies have

been performed in cancer models other than BC, the Warburg-like phenotype commonly observed in malignant breast tumours suggests that glucose restriction and lactic acidosis may be involved in the functional impairment of CTLs within the breast microenvironment. Furthermore, increased lactate production and acidification has been reported to impair not only T cells but also NK cells metabolism, again favouring immune escape and tumour growth [151].

The competition between cancer cells and TIL for glutamine may also contribute to hinder the protective role of TIL against BC progression. Indeed, high GLS expression in TNBC correlated with low levels of TIL [152]. On the other hand, glutaminase expression in TIL was significantly higher in cases with high levels of TIL. Hence, active glutamine consumption by tumour cells could account for glutamine deprivation of TIL, thus limiting their proliferation. Moreover, restriction of extracellular glutamine was reported to shift activation of CD4+ T cells towards a Treg phenotype, thus contributing to immune suppression *in vivo* [153]. Other amino acids with important roles in T cell survival and activation are arginine [154,155] and tryptophan [156,157]. Therefore, studying their shared use by cancer cells in the breast TME and knowing how immune effector and suppressor functions are modulated by the availability of these amino acids could potentially reveal additional therapeutic targets.

### *4.4. Metabolic interplay between myeloid-derived suppressor cells and breast cancer cells*

Metabolic reprogramming of MDSC plays an important role in BC progression. In mice bearing mammary 4T1 tumours, which are highly metastatic, massive accumulation of MDSC was accompanied by significant upregulation of glycolysis in these cells, as verified at the gene and mRNA levels and confirmed by *in vitro* coculture systems [158]. In particular, the glycolytic intermediate PEP was highlighted as a potent antioxidant metabolite, which prevented excessive ROS production and protected MDSC from ROS-induced apoptosis, thus contributing to their survival and expansion. In another study, the proteomic profile of MDSC from metastatic (4T1) and non-metastatic (67NR) BC mouse models revealed differential expression of several proteins related to platelet signalling, angiogenic response and cellular metabolism, namely glutathione, amino acid and lipid metabolisms [159]. In particular, spleen MDSC obtained from 4T1, but not from 67NR animals, displayed upregulation of γ-glutamyl transferases, transglutaminase, apolipoprotein E and ATPases, suggesting an association between the expression of these proteins in MDSC and the metastatic potential of the tumours.

### *4.5. Metabolic interplay between cancer-associated fibroblasts and breast cancer cells*

The metabolic coupling between BC cells and fibroblasts in the TME has been proposed about a decade ago and explained by the so-called reverse Warburg effect [160]. According to this model, stromal fibroblasts that are deficient in Caveolin 1 (Cav-1) display enhanced catabolism and produce metabolites (lactate, glutamine and ketones) that are used by neighbouring cancer cells to fuel oxidative energy production. In a series of studies, such metabolic reprogramming in CAF was demonstrated to be derived from the joint effect of upregulated glycolysis, mediated by HIF1α activation, together with autophagy induced by oxidative stress [161]. The differential metabolism of cocultured human CAF and BC cells was further demonstrated at the gene expression level, whereby cancer cells displayed upregulation in genes related to the TCA cycle and mitotic response, whereas CAF upregulated genes associated with increased glucose transport, glycolysis and glycogenesis [162]. Importantly, glycolytic CAF were shown to promote tumorigenesis both *in vitro* [163] and *in vivo* [164], as well as to induce anti-oestrogen resistance in ER-positive BC cells, by providing protection against stress-induced apoptosis [165]. These findings have motivated the modulation of the glucose-lactate shuttle in the TME to be assessed as a therapeutic strategy for patients with BC with low stromal Cav-1 expression. Promising results were obtained on treatment of a Cav-1-deficient xenograft murine model with the glycolytic inhibitors 2-deoxyglucose and dichloroacetate, which significantly reduced tumour growth [164].

The reverse Warburg effect underlying tumour–fibroblast interactions in the TME has also been reported in other cancer types, such as prostate cancer [166], colon carcinoma and melanoma [167]. However, opposing evidence has shown that this metabolic feature is not always present. Focussing on BC, Brauer et al have reported that the influence of CAF on BC cells glucose metabolism was actually dependent on the CAF phenotype [86]. While primary CAF isolated from luminal-like BC tissues suppressed glucose uptake by the luminal MCF7 BC cell line and had no effect on basal-like SUM149 cells, basal-like CAF induced enhanced glucose uptake in both BC cell lines. Furthermore, in a coculture system of claudin-low MDA-MB-231 BC cell line with CAF (obtained through stimulation of human bone marrow–derived mesenchymal stem cells with BC cells–conditioned medium), lactate derived from glycolytic tumour cells was found to be taken up by CAF [168,169]. Based on real-time polymerase chain reaction analysis and NMR spectroscopic measurements, this lactate was proposed to be transformed by CAF into metabolites such as pyruvate, which were then exported to fuel cancer cells

energetic and biosynthetic requirements [169]. Altogether, these studies demonstrate that interactions between cancer cells and CAF in regard to glucose metabolism may be dependent on factors such as stromal Cav-1 expression and tumour glycolytic activity, making it difficult to propose a single model to represent all BC cases.

The metabolic crosstalk between CAF and BC cells also appears to involve lipid metabolism and transport. Primary breast CAF have been recently reported to display overexpression and activity of FAS, leading to enhanced FA levels and secretion to external media [170]. On the other hand, BC cells incubated with CAF-conditioned medium presented decreased expression and activity of FAS, together with increased expression of the FA transporter, FATP1, and higher lipid levels. Hence, it was proposed that, under the influence of CAF, BC cells decreased lipid synthesis but enhanced their uptake from the microenvironment, thus suggesting lipid transport to be a possible target for modulating the BC cells–CAF interplay.

### *4.6. Metabolic interplay between adipocytes and breast cancer cells*

As mentioned previously, metabolic rewiring is one of the mechanisms through which adipocytes are considered to influence BC progression. This has been demonstrated in a recent study whereby, using coculture and conditioned media approaches, FFA released from adipocytes (differentiated from 3T3-L1 fibroblasts) were seen to feed BC cells (MDA-MB-231 or MCF-7) and to promote their proliferation and migration, while also altering their metabolism [171]. This effect was demonstrated to reflect adipocyte lipolysis mediated by hormone-sensitive lipase and adipose triglyceride lipase (ATGL), together with increased carnitine palmitoyltransferase (CPT1A) and electron transport chain complex protein levels in BC cells. Notably, obese adipocytes (3T3-L1 adipocyte cell line exposed to a lipid-rich environment) amplified this response in TNBC cells (MDA-MB-231), highlighting the role of direct metabolic substrate supply by adipocytes in obesity-mediated promotion of BC pathogenesis. The shuttling of FFA from adipocytes into tumour cells has been corroborated and further explored in another work, where FFA were seen to accumulate as triglycerides in lipid droplets of BC cells, both *in vitro* and *in vivo* [172]. This was followed by ATGL-dependent lipolysis and CPT1A-mediated translocation of FFA into the mitochondria of BC cells, causing FAO uncoupling, reduced ATP production and AMPK activation. These changes, observed when BC cells with low (ZR-75-1) and high (SUM159PT) invasive capacity were cocultured with adipocytes, were proposed to sustain the observed metabolic rewiring and to favour cell invasion. Indeed, inhibition of the ATGL-dependent lipolysis/FAO pathways was able to completely block tumour cells invasive capacity, highlighting ATGL as a possible therapeutic target against BC aggressiveness.

## 5. Conclusions

The TME plays a decisive role in BC progression, as shown by the prognostic value of specific cell populations, such as TIL, TAM or DC. Intermediary metabolism appears to be actively involved in the shaping of cell–cell interactions in the TME, directly contributing to either tumour-suppressive or tumour-promoting phenotypes. Metabolic studies of BC tissues and cells have identified alterations in several metabolic enzymes, fluxes and mediators, typically reflecting enhanced glycolysis, TCA cycle activity, glutaminolysis and lipid biosynthetic pathways. Such metabolic alterations vary according to the BC subtype and grade, while depending also on the complex dialogue between BC cells and the non-cancerous stromal cells present in the TME, such as fibroblasts, adipocytes, endothelial cells and several immune cells. Indeed, both competitive and symbiotic relationships have been found in BC TME, at the level of nutrient use and metabolic regulation. This intricate crosstalk appears to strongly influence tumour escape from immune surveillance mechanisms and to participate in cancer progression and metastasis. Hence, a deeper understanding of metabolic interactions in the TME has the potential to reveal vulnerabilities and pharmacological targets that may be explored in anticancer therapies.

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## Conflict of interest statement

None declared.

## References

[1] https://gco.iarc.fr, accessed 14 June 2019.
[2] https://www.cancer.org, accessed 14 June 2019.
[3] Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res 2015;5:2929–43.

[4] Nagarajan D, Mcardle SEB. Immune landscape of breast cancers. Biomedicines 2018;6:20. https://doi.org/10.3390/biomedicines6010020.
[5] Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 2008;14:518–27. https://doi.org/10.1038/nm1764.
[6] Farmer P, Bonnefoi H, Anderle P, Cameron D, Wirapati P, Becette V, et al. A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. Nat Med 2009;15:68–74. https://doi.org/10.1038/nm.1908.
[7] Pontiggia Olvaldo, Sampayo Rocio, Raffo Diego, Motter Andrea, Xu Ren, Bissel Mina J, et al. The tumor microenvironment modulates tamoxifen resistance in breast cancer: a role for soluble stromal factors and fibronectin through β1 integrin. Breast Canc Res Treat 2012;133:459–71. https://doi.org/10.1007/s10549-011-1766-x.
[8] Sampayo RG, Toscani AM, Rubashkin MG, Thi K, Masullo LA, Violi IL, et al. Fibronectin rescues estrogen receptor α from lysosomal degradation in breast cancer cells. J Cell Biol 2018;217. https://doi.org/10.1083/jcb.201703037.
[9] Park CC, Zhang HJ, Yao ES, Park CJ, Bissel MJ. Beta-1 integrin inhibition dramatically enhances radiotherapy efficacy in human breast cancer xenografts. Cancer Res 2008;68:4398–405. https://doi.org/10.1158/0008-5472.CAN-07-6390.
[10] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;4:646–74. https://doi.org/10.1016/j.cell.2011.02.013.
[11] Foud YA, Aanei C. Revisiting the hallmarks of cancer. Am J Cancer Res 2017;7:1016–36.
[12] Song E, Hu H. Translational research in breast cancer. Springer. Springer Nature; 2017.
[13] Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. Genes Dev 2018;32:1267–84. https://doi.org/10.1101/gad.314617.118.
[14] Seager RJ, Hajal C, Spill F, Kamm RD, Zaman MH. Dynamic interplay between tumour, stroma and immune system can drive or prevent tumour progression. Converg Sci Phys Oncol 2017;3. https://doi.org/10.1016/S0140-6736(01)22715-9.
[15] Azizi E, Carr AJ, Plitas G, Cornish AE, Konopacki C, Prabhakaran S, et al. Single-cell map of diverse immune phenotypes in the breast tumor microenvironment. Cell 2018;174:1–16. https://doi.org/10.1016/j.cell.2018.05.060.
[16] Chevrier S, Levine JH, Zanotelli VRT, Silina K, Schulz D, Bacac M, et al. An immune atlas of clear cell renal cell carcinoma. Cell 2017;169:736–49. https://doi.org/10.1016/j.cell.2017.04.016.
[17] Lavin Y, Kobayashi S, Leader A, Amir E ad D, Elefant N, Bigenwald C, et al. Innate immune landscape in early lung adenocarcinoma by paired single-cell analyses. Cell 2017;169:750–65. https://doi.org/10.1016/j.cell.2017.04.014.
[18] Zhang Q-W, Liu L, Gong C-Y, Shi H-S, Zeng Y-H, Wang X-Z, et al. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. PLoS One 2012;7:1–14. https://doi.org/10.1371/journal.pone.0050946.
[19] Makela AV, Foster PJ. Imaging macrophage distribution and density in mammary tumors and lung metastases using fluorine-19 MRI cell tracking. Magn Reson Med 2018;80:1138–47. https://doi.org/10.1002/mrm.27081.
[20] Aras S, Zaidi MR. TAMeless traitors: macrophages in cancer progression and metastasis. Br J Canc 2017;117:1583–91. https://doi.org/10.1038/bjc.2017.356.
[21] Tiainen S, Tumelius R, Rilla K, Hämäläinen K, Tammi M, Tammi R, et al. High numbers of macrophages, especially M2-like (CD163-positive), correlate with hyaluronan accumulation and poor outcome in breast cancer. Histopathology 2015;66:873–83. https://doi.org/10.1111/his.12607.
[22] Medrek C, Pontén F, Jirström K, Leandersson K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BioMed Cent 2012;12:1. https://doi.org/10.1186/1471-2407-12-306.
[23] Sousa S, Brion R, Lintunen M, Kronqvist P, Sandholm J, Mönkkönen J, et al. Human breast cancer cells educate macrophages toward the M2 activation status. Breast Cancer Res 2015;17:1–14. https://doi.org/10.1186/s13058-015-0621-0.
[24] Yang M, Li Z, Ren M, Li S, Zhang L, Zhang X, et al. Stromal infiltration of tumor-associated macrophages conferring poor prognosis of patients with basal-like breast carcinoma. J Cancer 2018;9:2308–16. https://doi.org/10.7150/jca.25155.
[25] Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Worthen GS, et al. Polarization of TAN phenotype by TGFb: N1 versus N2 TAN. Cancer Cell 2009;16:183–94. https://doi.org/10.1016/j.ccr.2009.06.017.
[26] Shen M, Hu P, Donskov F, Wang G, Liu Q, Du J. Tumor-associated neutrophils as a new prognostic factor in cancer: a systematic review and meta-analysis. PLoS One 2014;9:1–10. https://doi.org/10.1371/journal.pone.0098259.
[27] Berry RS, Xiong MJ, Greenbaum A, Mortaji P, Nofchissey RA, Schultz F, et al. High levels of tumor-associated neutrophils are associated with improved overall survival in patients with stage II colorectal cancer. PLoS One 2017;12:1–12. https://doi.org/10.1371/journal.pone.0188799.
[28] Soto-Perez-de-Celis E, Chavarri-Guerra Y, Leon-Rodriguez E, Gamboa-Dominguez A. Tumor-associated neutrophils in breast cancer subtypes. Asian Pac J Cancer Prev APJCP 2017;18:2689–93. https://doi.org/10.22034/APJCP.2017.18.10.2689.
[29] Veglia F, Gabrilovich DI. Dendritic cells in cancer: the role revisited. Curr Opin Immunol 2017;45:43–51. https://doi.org/10.1016/j.coi.2017.01.002.
[30] Treilleux I, Blay JY, Bendriss-Vermare N, Ray-Coquard I, Bachelot T, Guastolla JP, et al. Dendritic cell infiltration and prognosis of early stage breast cancer. Clin Cancer Res 2004;10:7466–74. https://doi.org/10.1158/1078-0432.CCR-04-0684.
[31] Bailur JK, Gueckel B, Pawelec G. Prognostic impact of high levels of circulating plasmacytoid dendritic cells in breast cancer. J Transl Med 2016;14:1–10. https://doi.org/10.1186/s12967-016-0905-x.
[32] Sisirak V, Faget J, Gobert M, Goutagny N, Vey N, Treilleux I, et al. Impaired IFN-α production by plasmacytoid dendritic cells favors regulatory T-cell expansion that may contribute to breast cancer progression. Cancer Res 2012;72:5188–97. https://doi.org/10.1158/0008-5472.CAN-11-3468.
[33] Michea P, Noël F, Zakine E, Czerwinska U, Sirven P, Abouzid O, et al. Adjustment of dendritic cells to the breast-cancer microenvironment is subset specific. Nat Immunol 2018;19:885–97. https://doi.org/10.1038/s41590-018-0145-8.
[34] Leong PP, Mohammad R, Ibrahim N, Ithnin H, Abdullah M, Davis WC, et al. Phenotyping of lymphocytes expressing regulatory and effector markers in infiltrating ductal carcinoma of the breast. Immunol Lett 2006;102:229–36. https://doi.org/10.1016/j.imlet.2005.09.006.
[35] Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 2013;31:860–7. https://doi.org/10.1200/JCO.2011.41.0902.
[36] Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol 2014;32:2959–66. https://doi.org/10.1200/JCO.2013.55.0491.

[37] Mao Y, Qu Q, Chen X, Huang O, Wu J, Shen K. The prognostic value of tumor-infiltrating lymphocytes in breast cancer: a systematic review and meta-analysis. PLoS One 2016;11:1–13. https://doi.org/10.1371/journal.pone.0152500.

[38] Russo L, Maltese A, Betancourt L, Romero G, Cialoni D, De la Fuente L, et al. Locally advanced breast cancer: tumor-infiltrating lymphocytes as a predictive factor of response to neoadjuvant chemotherapy. Eur J Surg Oncol 2019;45:963–8. https://doi.org/10.1016/j.ejso.2019.01.222.

[39] Ogiya R, Niikura N, Kumaki N, Bianchini G, Kitano S, Iwamoto T, et al. Comparison of tumor-infiltrating lymphocytes between primary and metastatic tumors in breast cancer patients. Cancer Sci 2016;107:1730–5. https://doi.org/10.1111/cas.13101.

[40] Oldford SA, Robb JD, Codner D, Gadag V, Watson PH, Drover S. Tumor cell expression of HLA-DM associates with a Th1 profile and predicts improved survival in breast carcinoma patients. Int Immunol 2006;18:1591–602. https://doi.org/10.1093/intimm/dxl092.

[41] Zhang Q, Qin J, Zhong L, Gong L, Zhang B, Zhang Y, et al. CCL5-Mediated Th2 immune polarization promotes metastasis in luminal breast cancer. Cancer Res 2015;75:4312–21. https://doi.org/10.1158/0008-5472.CAN-14-3590.

[42] Yang LJ, Qi YX, Hu J, Tang L, Zhao S, Shan BE. Expression of Th17 cells in breast cancer tissue and its association with clinical parameters. Cell Biochem Biophys 2012;62:153–9. https://doi.org/10.1007/s12013-011-9276-3.

[43] Xu L, Xu W, Qiu S, Xiong S. Enrichment of CCR6+Foxp3+regulatory T cells in the tumor mass correlates with impaired CD8+T cell function and poor prognosis of breast cancer. Clin Immunol 2010;135:466–75. https://doi.org/10.1016/j.clim.2010.01.014.

[44] Khaja A, Toor SM, Salhat H El, Faour I, Haq NU, Ali BR, et al. Preferential accumulation of regulatory T cells with highly immunosuppressive characteristics in breast tumor microenvironment. Oncotarget 2017;8:33159–71. https://doi.org/10.18632/oncotarget.16565.

[45] Bates GJ, Fox SB, Han C, Leek RD, Garcia JF, Harris AL, et al. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. J Clin Oncol 2006;24:5373–80. https://doi.org/10.1200/JCO.2006.05.9584.

[46] Knochelmann HM, Dwyer CJ, Bailey SR, Amaya SM, Elston DM, Mazza-McCrann JM, et al. When worlds collide: Th17 and Treg cells in cancer and autoimmunity. Cell Mol Immunol 2018;15:458–69.

[47] Perez SA, Karamouzis MV, Skarlos DV, Ardavanis A, Sotiriadou NN, Iliopoulou EG, et al. CD4+CD25+ regulatory T-cell frequency in HER-2/neu (HER)-positive and HER-negative advanced-stage Breast Cancer Patients. Clin Cancer Res 2007;13:2714–21. https://doi.org/10.1158/1078-0432.CCR-06-2347.

[48] Li Y, Sun R. Tumor immunotherapy: new aspects of natural killer cells. Chin J Canc Res 2018;30:173–96. https://doi.org/10.21147/j.issn.1000-9604.2018.02.02.

[49] Verma C, Kaewkangsadan V, Eremin JM, Cowley GP, Ilyas M, El-Sheemy MA, et al. Natural killer (NK) cell profiles in blood and tumour in women with large and locally advanced breast cancer (LLABC) and their contribution to a pathological complete response (PCR) in the tumour following neoadjuvant chemotherapy (NAC): differential restoration of blood profiles by NAC and surgery. J Transl Med 2015;13:1–21. https://doi.org/10.1186/s12967-015-0535-8.

[50] Mariel GC, Edith CTI, Pilar CR, Elena GDN, Humberto RM, Guadalupe MSM, et al. Expression of NK cell surface receptors in breast cancer tissue as predictors of resistance to antineoplastic treatment. Technol Cancer Res Treat 2018;17:1–11. https://doi.org/10.1177/1533033818764499.

[51] Mamessier E, Sylvain A, Bertucci F, Castellano R, Finetti P, Houvenaeghel G, et al. Human breast tumor cells induce self-tolerance mechanisms to avoid NKG2D-mediated and DNAM-mediated NK cell recognition. Cancer Res 2011;71:6621–32. https://doi.org/10.1158/0008-5472.CAN-11-0792.

[52] Algarra I, García-Lora A, Cabrera T, Ruiz-Cabello F, Garrido F. The selection of tumor variants with altered expression of classical and nonclassical MHC class I molecules: implications for tumor immune escape. Cancer Immunol Immunother 2004;53:904–10. https://doi.org/10.1007/s00262-004-0517-9.

[53] de Kruijf EM, Sajet A, van Nes JGH, Natanov R, Putter H, Smit VTHBM, et al. HLA-E and HLA-G expression in classical HLA class I-negative tumors is of prognostic value for clinical outcome of early breast cancer patients. J Immunol 2010;185:7452–9. https://doi.org/10.4049/jimmunol.1002629.

[54] Umansky V, Blattner C, Gebhardt C, Utikal J. The role of myeloid-derived suppressor cells (MDSC) in cancer progression. Vaccines 2016;4:36. https://doi.org/10.3390/vaccines4040036.

[55] Safarzadeh E, Hashemzadeh S, Duijf PHG, Mansoori B, Khaze V, Mohammadi A, et al. Circulating myeloid-derived suppressor cells: an independent prognostic factor in patients with breast cancer. J Cell Physiol 2018;234:3515–25. https://doi.org/10.1002/jcp.26896.

[56] Diaz-Montero C, Salem M, Nishimura M, Garrett-Mayer E, Cole D, Montero A. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother 2009;58:49–59. https://doi.org/10.1007/s00262-008-0523-4.

[57] Montero A, Diaz-Montero C, Deutsch Y, Hurley J, Koniaris L, Rumboldt T, et al. Phase 2 Study of neoadjuvant treatment with Nov-002 in combination with doxorubicin and cyclophosphamide followed by docetaxel in patients with HER-2 negative clinical stage II- IIIc breast cancer. Breast Canc Res Treat 2012;132:215–23. https://doi.org/10.1007/s10549-011-1889-0.

[58] Gonda K, Shibata M, Ohtake T, Matsumoto Y, Tachibana K, Abe N, et al. Myeloid-derived suppressor cells are increased and correlated with type 2 immune responses, malnutrition, inflammation, and poor prognosis in patients with breast cancer. Oncol Lett 2017;14:1766–74. https://doi.org/10.3892/ol.2017.6305.

[59] Mittal S, Brown NJ, Holen I. The breast tumor microenvironment: role in cancer development, progression and response to therapy. Expert Rev Mol Diagn 2018;18:227–43. https://doi.org/10.1080/14737159.2018.1439382.

[60] Xing F, Saidou J, Watabe K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. Front Biosci 2011;15:166–79. https://doi.org/10.1080/00387010.2016.1211708.

[61] Eiro N, González L, Martínez-Ordoñez A, Fernandez-Garcia B, González LO, Cid S, et al. Cancer-associated fibroblasts affect breast cancer cell gene expression, invasion and angiogenesis. Cell Oncol 2018;41:369–78. https://doi.org/10.1007/s13402-018-0371-y.

[62] Hu G, Xu F, Zhong K, Wang S, Huang L, Chen W. Activated tumor-infiltrating fibroblasts predict worse prognosis in breast cancer patients. J Cancer 2018;9:3736–42. https://doi.org/10.7150/jca.28054.

[63] Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M, et al. Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. Cancer Cell 2018;33:463–79. https://doi.org/10.1016/j.ccell.2018.01.011.

[64] Picon-Ruiz M, Morata-Tarifa C, Valle-Goffin JJ, Friedman ER, Slingerland JM. Obesity and adverse breast cancer risk and outcome: mechanistic insights and strategies for intervention. CA A Cancer J Clin 2017;67:378–97. https://doi.org/10.3322/caac.21405.

[65] Choi J, Jin Y, Seung J. Progress in lipid research adipocyte biology in breast cancer: from silent bystander to active

facilitator. Prog Lipid Res 2018;69:11–20. https://doi.org/10.1016/j.plipres.2017.11.002.
[66] Dirat B, Bochet L, Dabek M, Daviaud D, Dauvillier S, Majed B, et al. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer Res 2011; 71:2455–65. https://doi.org/10.1158/0008-5472.CAN-10-3323.
[67] Picon-Ruiz M, Pan C, Drews-Elger K, Jang K, Besser AH, Zhao D, et al. Interactions between adipocytes and breast cancer cells stimulate cytokine production and drive Src/Sox2/miR-302b-mediated malignant progression. Cancer Res 2016;76: 491–504. https://doi.org/10.1158/0008-5472.CAN-15-0927.
[68] Nickel A, Blücher C, Kadri O Al, Schwagarus N, Müller S, Schaab M, et al. Adipocytes induce distinct gene expression profiles in mammary tumor cells and enhance inflammatory signaling in invasive breast cancer cells. Sci Rep 2018;8:1–13. https://doi.org/10.1038/s41598-018-27210-w.
[69] Lehuédé C, Li X, Dauvillier S, Vaysse C, Franchet C, Clement E, et al. Adipocytes promote breast cancer resistance to chemotherapy, a process amplified by obesity: role of the major vault protein (MVP). Breast Cancer Res 2019;21:1–17. https://doi.org/10.1186/s13058-018-1088-6.
[70] Buchanan CF, Szot CS, Wilson TD, Akman S, Metheny-Barlow LJ, Robertson JL, et al. Cross-talk between endothelial and breast cancer cells regulates reciprocal expression of angiogenic factors in vitro. J Cell Biochem 2012;113:1142–51. https://doi.org/10.1002/jcb.23447.
[71] Ghiabi P, Jiang J, Pasquier J, Maleki M, Abu-Kaoud N, Rafii S, et al. Endothelial cells provide a notch-dependent pro-tumoral niche for enhancing breast cancer survival, stemness and pro-metastatic properties. PLoS One 2014;9:e112424. https://doi.org/10.1371/journal.pone.0112424.
[72] Zhang W, Xu J, Fang H, Tang L, Chen W, Sun Q, et al. Endothelial cells promote triple-negative breast cancer cell metastasis via PAI-1 and CCL5 signaling. FASEB J 2018;32: 276–88. https://doi.org/10.1096/fj.201700237RR.
[73] 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;13:1. https://doi.org/10.1186/1471-2164-13-334.
[74] Tayyari F, Yang HH, Ngwa WF, Mittal SK, Raftery D, Lee MP, et al. Metabolic profiles of triple-negative and luminal A breast cancer subtypes in African-American identify key metabolic differences. Oncotarget 2018;9:11677–90. https://doi.org/10.18632/oncotarget.24433.
[75] More TH, RoyChoudhury S, Christie J, Taunk K, Mane A, Santra MK, et al. Metabolomic alterations in invasive ductal carcinoma of breast: a comprehensive metabolomic study using tissue and serum samples. Oncotarget 2018;9:2678–96. https://doi.org/10.18632/oncotarget.23626.
[76] Sitter B, Lundgren S, Bathen TF, Halgunset J, Fjosne HE, Gribbestad IS. Comparison of HR MAS MR spectroscopic profiles of breast cancer tissue with clinical parameters. NMR Biomed 2006;19:30–40. https://doi.org/10.1002/nbm.992.
[77] Giskeødegård GF, Grinde MT, Sitter B, Axelson DE, Lundgre S, Fjøsne HE, et al. Multivariate modeling and prediction of breast cancer prognostic factor using MR metabolomics. J Proteome Res 2010;9:927–79. https://doi.org/10.1021/ja02259a024.
[78] Tang X, Lin CC, Spasojevic I, Iversen ES, Chi JT, Marks JR. A joint analysis of metabolomics and genetics of breast cancer. Breast Cancer Res 2014;16:1–15. https://doi.org/10.1186/s13058-014-0415-9.
[79] Haukaas TH, Euceda LR, Giskeødegård GF, Lamichhane S, Krohn M, Jernström S, et al. Metabolic clusters of breast cancer in relation to gene- and protein expression subtypes. Cancer Metabol 2016;4:1–14. https://doi.org/10.1186/s40170-016-0152-x.
[80] Lane AN, Tan J, Wang Y, Yan J, Higashi RM, Fan TWM. Probing the metabolic phenotype of breast cancer cells by multiple tracer stable isotope resolved metabolomics. Metab Eng 2017;43:125–36. https://doi.org/10.1016/j.ymben.2017.01.010.
[81] Dubuis S, Baenke F, Scherbichler N, Alexander LT, Schulze A, Zamboni N. Metabotypes of breast cancer cell lines revealed by non-targeted metabolomics. Metab Eng 2017;43:173–86. https://doi.org/10.1016/j.ymben.2016.12.009.
[82] Willmann L, Schlimpert M, Halbach S, Erbes T, Stickeler E, Kammerer B. Metabolic profiling of breast cancer: differences in central metabolism between subtypes of breast cancer cell lines. J Chromatogr B 2015;1000:95–104. https://doi.org/10.1016/j.jchromb.2015.07.021.
[83] Willmann L, Schlimpert M, Hirschfeld M, Erbes T, Neubauer H, Stickeler E, et al. Alterations of the exo- and endometabolite profiles in breast cancer cell lines: a mass spectrometry-based metabolomics approach. Anal Chim Acta 2016;925:34–42. https://doi.org/10.1016/j.aca.2016.04.047.
[84] Liberti MV, Locasale JW. Review – the Warburg effect : how does it benefit cancer cells? Trends Biochem Sci 2016;41:211–8. https://doi.org/10.1016/j.tibs.2015.12.001.
[85] Zhu A, Lee D, Shim H. Metabolic PET imaging in cancer detection and therapy response. Semin Oncol 2011;38:55–69. https://doi.org/10.1053/j.seminoncol.2010.11.012. Metabolic.
[86] Brauer HA, Makowski L, Hoadley KA, Hernandez PC-H, Lang LJ, Romàn-Pèrez E, et al. Impact of tumor and epithelial phenotypes on metabolism in breast cancer. Clin Cancer Res 2013;19:571–85. https://doi.org/10.1158/1078-0432.CCR-12-2123.
[87] Lanning NJ, Castle JP, Singh SJ, Leon AN, Tovar EA, Sanghera A, et al. Metabolic profiling of triple-negative breast cancer cells reveals metabolic vulnerabilities. Cancer Metabol 2017;5:1–14. https://doi.org/10.1186/s40170-017-0168-x.
[88] Wang J, Ye C, Chen C, Xiong H, Xie B, Zhou J, et al. Glucose transporter GLUT1 expression and clinical outcome in solid tumors: a systematic review and meta-analysis. Oncotarget 2017; 8:16875–86. https://dx.doi.org/10.18632/oncotarget.15171.
[89] Choi J, Jung WH, Koo JS. Metabolism-related proteins are differentially expressed according to the molecular subtype of invasive breast cancer defined by surrogate immunohistochemistry. Pathobiology 2013;80:41–52. https://doi.org/10.1159/000339513.
[90] Krzeslak A, Wojcik-Krowiranda K, Forma E, Jozwiak P, Romanowicz H, Bienkiewicz A, et al. Expression of GLUT1 and GLUT3 glucose transporters in endometrial and breast cancers. Pathol Oncol Res 2012;18:721–8. https://doi.org/10.1007/s12253-012-9500-5.
[91] Pedersen PL, Mathupala S, Rempel A, Geschwind JF, Ko YH. Mitochondrial bound type II hexokinase: a key player in the growth and survival of many cancers and an ideal prospect for therapeutic intervention. Biochim Biophys Acta Bioenerg 2002;1555:14–20. https://doi.org/10.1016/S0005-2728(02)00248-7.
[92] Brown RS, Goodman TM, Zasadny KR, Greenson JK, Wahl RL. Expression of hexokinase II and Glut-1 in untreated human breast cancer. Nucl Med Biol 2002;29:443–53. https://doi.org/10.1016/S0969-8051(02)00288-3.
[93] Gudnason V, Ingvarsson Si, Jonasdottir A, Andresdottir V, Egilsson V. Isoenzyme pattern and subcellular localization of hexokinases in human breast cancer and nonpathological breast tissue. Int J Cancer 1984;34:63–6. https://doi.org/10.1002/ijc.2910340111.
[94] Patra KC, Wang Q, Bhaskar PT, Miller L, Wang Z, Chandel N, et al. Hexokinase 2 is requried for tumor initiation and maintenance in mouse models of cancer. Cancer Cell 2013;24:213–28. https://doi.org/10.1016/j.ccr.2013.06.014.

[95] Hennipman A, Smits J, Oirschot B, Houwellingen JC, Rijsen G, Neyt JP, et al. Glycolytic enzymes in breast cancer, benign breast disease and normal breast tissue. Tumor Biol 1987;8:251–63.
[96] Wang G, Xu Z, Wang C, Yao F, Li J, Chen C, et al. Differential phosphofructokinase-1 isoenzyme patterns associated with glycolytic efficiency in human breast cancer and paracancer tissues. Oncol Lett 2013;6:1701–6. https://doi.org/10.3892/ol.2013.1599.
[97] Dong G, Mao Q, Xia W, Xu Y, Wang J, Xu L, et al. PKM2 and cancer: the function of PKM2 beyond glycolysis. Oncol Lett 2016;11. https://doi.org/10.3892/ol.2016.4168. 1980–6.
[98] Yang Y, Wu K, Liu Y, Shi L, Tao K, Wang G. Prognostic significance of metabolic enzyme pyruvate kinase M2 in breast cancer: a meta-analysis. Medicine 2017;96:1–7. https://doi.org/10.1097/MD.0000000000008690.
[99] Hall MJ, Reid JE, Burbidge LA, Pruss D, Deffenbaugh AM, Frye C, et al. BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. Cancer 2009;115:2222–33. https://doi.org/10.1002/cncr.24200.
[100] Privat M, Radosevic-Robin N, Aubel C, Cayre A, Penault-Llorca F, Marceau G, et al. BRCA1 induces major energetic metabolism reprogramming in breast cancer cells. PLoS One 2014;9:e102438. https://doi.org/10.1371/journal.pone.0102438.
[101] Zhao YH, Zhou M, Liu H, Khong HT, Yu D, Fodstad O, et al. Upregulation of lactate dehydrogenase A by ErbB2 through heat shock factor 1 promotes breast cancer cell glycolysis and growth. Oncogene 2009;28:3689–701. https://doi.org/10.1038/onc.2009.229.
[102] Semenza GL. Hypoxia-inducible factors: coupling glucose metabolism and redox regulation with induction of the breast cancer stem cell phenotype. EMBO J 2017;36:252–9. https://doi.org/10.15252/embj.201695204.
[103] Chiavarina B, Martinez-Outschoorn UE, Whitaker-Menezes D, Howell A, Tanowitz HB, Lisanti MP, et al. Metabolic reprogramming and two-compartment tumor metabolism. Cell Cycle 2012;11:3280–9. https://doi.org/10.4161/cc.21643.
[104] Dupuy F, Tabariès S, Andrzejewski S, Dong Z, Blagih J, Annis MG, et al. PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. Cell Metabol 2015;22:577–89. https://doi.org/10.1016/j.cmet.2015.08.007.
[105] Yang L, Hou Y, Yuan J, Tang S, Zhang H, Zhu Q, et al. Twist promotes reprogramming of glucose metabolism in breast cancer cells through PI3K/AKT and p53 signaling pathways. Oncotarget 2015;6:25755–69. https://doi.org/10.18632/oncotarget.4697.
[106] Ahn CS, Metallo CM. Mitochondria as biosynthetic factories for cancer proliferation. Cancer Metabol 2015;3:1–10. https://doi.org/10.1186/s40170-015-0128-2.
[107] Santidrian AF, Matsuno-Yagi A, Ritland M, Seo BB, LeBoeuf SE, Gay LJ, et al. Mitochondrial complex I activity and NAD+/NADH balance regulate breast cancer progression. J Clin Investig 2013;123:1068–81. https://doi.org/10.1172/JCI64264.
[108] Imanishi H, Hattori K, Wada R, Ishikawa K, Fukuda S, Takenaga K, et al. Mitochondrial DNA mutations regulate metastasis of human breast cancer cells. PLoS One 2011;6:1–7. https://doi.org/10.1371/journal.pone.0023401.
[109] Eastlack SC, Dong S, Ivan C, Alahari SK. Suppression of PDHX by microRNA-27b deregulates cell metabolism and promotes growth in breast cancer. Mol Cancer 2018;17:1–16. https://doi.org/10.1186/s12943-018-0851-8.
[110] Kim HM, Jung WH, Koo JS. Site-specific metabolic phenotypes in metastatic breast cancer. J Transl Med 2014;12:1–17. https://doi.org/10.1186/s12967-014-0354-3.
[111] Kim S, Kim DH, Jung WH, Koo JS. Succinate dehydrogenase expression in breast cancer. SpringerPlus 2013;2:1–12. https://doi.org/10.1186/2193-1801-2-299.
[112] Hensley CT, Wasti AT, Deberardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. J Clin Investig 2013;123:3678–84. https://doi.org/10.1172/JCI69600.3678.
[113] Kim S, Kim DH, Jung WH, Koo JS. Expression of glutamine metabolism-related proteins according to molecular subtype of breast cancer. Endocr Relat Cancer 2013;20:339–48. https://doi.org/10.1530/ERC-12-0398.
[114] Lampa M, Arlt H, He T, Ospina B, Reeves J, Zhang B, et al. Glutaminase is essential for the growth of triple-negative breast cancer cells with a deregulated glutamine metabolism pathway and its suppression synergizes with mTOR inhibition. PLoS One 2017;12. https://doi.org/10.1371/journal.pone.0185092.
[115] Kung HN, Marks JR, Chi JT. Glutamine synthetase is a genetic determinant of cell type-specific glutamine independence in breast epithelia. PLoS Genet 2011;7. https://doi.org/10.1371/journal.pgen.1002229.
[116] Mattaini KR, Sullivan MR, Vander Heiden MG. The importance of serine metabolism in cancer. J Cell Biol 2016;214:249–57. https://doi.org/10.1083/jcb.201604085.
[117] Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Sethumadhavan S, et al. Functional genomics reveals serine synthesis is essential in PHGDH-amplified breast cancer. Nature 2012;476:346–50. https://doi.org/10.1038/nature10350.
[118] 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 2013;43:869–74. https://doi.org/10.1038/ng.890.Phosphoglycerate.
[119] Chen J, Chung F, Yang G, Pu M, Gao H, Jiang W, et al. Phosphoglycerate dehydrogenase is dispensable for breast tumor maintenance and growth. Oncotarget 2013;4:2502–11. https://doi.org/10.18632/oncotarget.1540.
[120] Yang M, Vousden KH. Serine and one-carbon metabolism in cancer. Nat Rev Cancer 2016;16:650–62. https://doi.org/10.1038/nrc.2016.81.
[121] Fan J, Teng X, Liu L, Looper R, Rabinowitz J. Human phosphoglycerate dehydrogenase produces the oncometabolite D-2-hydroxyglutarate and promotes histone methylation. Cancer Metabol 2015;2:510–6. https://doi.org/10.1186/2049-3002-2-S1-P75.
[122] Currie E, Schulze A, Zechner R, Walther TC, Farese RV. Cellular fatty acid metabolism and cancer. Cell Metabol 2013;18:153–61. https://doi.org/10.1016/j.cmet.2013.05.017.
[123] Hilvo M, Denkert C, Lehtinen L, Müller B, Brockmöller S, Seppänen-Laakso T, et al. Novel theranostic opportunities offered by characterization of altered membrane lipid metabolism in breast cancer progression. Cancer Res 2011;71:3236–45. https://doi.org/10.1158/0008-5472.CAN-10-3894.
[124] Balaban S, Lee LS, Varney B, Aishah A, Gao Q, Shearer RF, et al. Heterogeneity of fatty acid metabolism in breast cancer cells underlies differential sensitivity to palmitate-induced apoptosis. Mol Oncol 2018;12:1623–38. https://doi.org/10.1002/1878-0261.12368.
[125] Menendez JA, Lupu R. Fatty acid synthase (FASN) as a therapeutic target in breast cancer. Expert Opin Ther Targets 2017;21:1001–16. https://doi.org/10.1080/14728222.2017.1381087.
[126] Kim S, Lee Y, Koo JS. Differential expression of lipid metabolism-related proteins in different breast cancer subtypes. PLoS One 2015;10:1–15. https://doi.org/10.1371/journal.pone.0119473.
[127] Jin Q, Yuan LX, Boulbes D, Baek JM, Wang YN, Gomez-Cabello D, et al. Fatty acid synthase phosphorylation: a novel therapeutic target in HER2-overexpressing breast cancer cells. Breast Cancer Res 2010;12:1–15. https://doi.org/10.1186/bcr2777.

[128] Jain M, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, et al. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. Science 2012;336:1040–4. https://doi.org/10.1126/science.1218595.

[129] Katz-Brull R, Margalit R, Bendel P, Degani H. Choline metabolism in breast cancer; 2H-, 13C- and 31P-NMR studies of cells and tumors. Magn Reson Mater Physics. Biol Med 1998;6:44–52.

[130] Glunde K, Jie C, Bhujwalla ZM. Molecular causes of the aberrant choline phospholipid metabolism in breast cancer. Cancer Res 2004;64:4270–6. https://doi.org/10.1158/0008-5472.CAN-03-3829.

[131] Noh DY, Ahn SJ, Lee RA, Park IA, Kim JH, Suh PG, et al. Overexpression of phospholipase D1 in human breast cancer tissues. Cancer Lett 2000;161:207–14. https://doi.org/10.1016/S0304-3835(00)00612-1.

[132] Chen Y, Zheng Y, Foster DA. Phospholipase D confers rapamycin resistance in human breast cancer cells. Oncogene 2003;22:3937–42. https://doi.org/10.1038/sj.onc.1206565.

[133] Sonveaux P, Végran F, Schroeder T. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J Clin Investig 2008;18:3930–42. https://doi.org/10.1172/JCI36843.

[134] Pisarsky L, Bill R, Fagiani E, Dimeloe S, Ryan G, Hagmannm J, et al. Targeting metabolic symbiosis to overcome resistance to anti-angiogenic therapy. Cell Rep 2016;15:1161–74. https://doi.org/10.1016/J.celrep.2016.04.028.

[135] Kennedy KM, Scarbrough PM, Ribeiro A, Richardson R, Yuan H, Sonveaux P, et al. Catabolism of exogenous lactate reveals it as a legitimate metabolic substrate in breast cancer. PLoS One 2013;8. https://doi.org/10.1371/journal.pone.0075154.

[136] Lee DC, Sohn HA, Park ZY, Oh S, Kang YK, Lee KM, et al. A lactate-induced response to hypoxia. Cell 2015;161:595–609. https://doi.org/10.1016/j.cell.2015.03.011.

[137] Rabold K, Netea MG, Adema GJ, Netea-Maier RT. Cellular metabolism of tumor-associated macrophages – functional impact and consequences. FEBS Lett 2017;591:3022–41. https://doi.org/10.1002/1873-3468.12771.

[138] Liu D, Chang C, Lu N, Wang X, Lu Q, Ren X, et al. Comprehensive proteomics analysis reveals metabolic reprogramming of tumor-associated macrophages stimulated by the tumor microenvironment. J Proteome Res 2017;16:288–97. https://doi.org/10.1021/acs.jproteome.6b00604.

[139] Wenes M, Shang M, Di Matteo M, Goveia J, Martín-Pérez R, Serneels J, et al. Macrophage metabolism controls tumor blood vessel morphogenesis and metastasis. Cell Metabol 2016;24:701–15. https://doi.org/10.1016/j.cmet.2016.09.008.

[140] Lin S, Sun L, Lyu X, Ai X, Du D, Su N, et al. Lactate-activated macrophages induced aerobic glycolysis and epithelial-mesenchymal transition in breast cancer by regulation of CCL5-CCR5 axis: a positive metabolic feedback loop. Oncotarget 2017;8:110426–43. https://doi.org/10.18632/oncotarget.22786.

[141] Chen P, Zuo H, Xiong H, Kolar MJ, Chu Q, Saghatelian A, et al. Gpr132 sensing of lactate mediates tumor–macrophage interplay to promote breast cancer metastasis. Proc Natl Acad Sci 2017;114:580–5. https://doi.org/10.1073/pnas.1614035114.

[142] Hollmén M, Roudnicky F, Karaman S, Detmar M. Characterization of macrophage - cancer cell crosstalk in estrogen receptor positive and triple-negative breast cancer. Sci Rep 2015;5:1–10. https://doi.org/10.1038/srep09188.

[143] Rath M, Müller I, Kropf P, Closs EI, Munder M. Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. Front Immunol 2014;5:1–10. https://doi.org/10.3389/fimmu.2014.00532.

[144] DiNapoli MR, Calderon CL, Lopez DM. The altered tumoricidal capacity of macrophages isolated from tumor- bearing mice is related to reduce expression of the inducible nitric oxide synthase gene. J Exp Med 1996;183:1323–9. https://doi.org/10.1084/jem.183.4.1323.

[145] Bantug GR, Galluzzi L, Kroemer G, Hess C. The spectrum of T cell metabolism in health and disease. Nat Rev Immunol 2018;18:19–34. https://doi.org/10.1038/nri.2017.99.

[146] Chang C, Qiu J, Sullivan DO, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell 2015;162:1229–41. https://doi.org/10.1016/j.cell.2015.08.016.

[147] Ho P, Bihuniak JD, Macintyre AN, Staron M, Amezquita R, Tsui Y, et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. Cell 2016;162:1217–28. https://doi.org/10.1016/j.cell.2015.08.012.

[148] Fischer K, Edinger M, Gottfried E, Andreesen R, Timischl B, Hoffmann P, et al. Inhibitory effect of tumor cell-derived lactic acid on human T cells. Blood 2007;109:3812–9. https://doi.org/10.1182/blood-2006-07-035972.

[149] Mendler AN, Hu B, Prinz P, Kreutz M, Gottfried E, Noessner E. Tumor lactic acidosis suppresses CTL function by inhibition of p38 and JNK/c-Jun activation. Int J Cancer 2011;131:633–40. https://doi.org/10.1002/ijc.26410.

[150] Calcinotto A, Filipazzi P, Grioni M, Iero M, Milito A, Ricupito A, et al. Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. Microenviron Immunol 2012;72:2746–56. https://doi.org/10.1158/0008-5472.can-11-1272.

[151] Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, et al. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. Cell Metabol 2016;24:657–71. https://doi.org/10.1016/j.cmet.2016.08.011.

[152] Kim JY, Heo SH, Choi SK, Song IH, Park IA, Kim YA, et al. Glutaminase expression is a poor prognostic factor in node-positive triple-negative breast cancer patients with a high level of tumor-infiltrating lymphocytes. Virchows Arch 2017;470:381–9. https://doi.org/10.1007/s00428-017-2083-5.

[153] Klysz D, Tai X, Robert P, Craveiro M, Cretenet G, Oburoglu L, et al. Glutamine-dependent α-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. Immunology 2015;8:ra97. https://doi.org/10.1126/scisignal.aab2610.

[154] Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-Arginine modulates T cell metabolism and enhances survival and anti-tumor activity. Cell 2016;167:829–42. https://doi.org/10.1016/j.cell.2016.09.031. e13.

[155] Rodriguez PC, Quiceno DG, Ochoa AC. L-arginine availability regulates T-lymphocyte cell-cycle progression. Blood J 2007;109:1568–74. https://doi.org/10.1182/blood-2006-06-031856.

[156] Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med 1999;189:1363–72. https://doi.org/10.7763/IJCEE.2014.V6.790.

[157] Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, et al. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor-chain and induce a regulatory phenotype in naive T cells. J Immunol 2006;176:6752–61. https://doi.org/10.4049/jimmunol.176.11.6752.

[158] Jian SL, Chen WW, Su YC, Su YW, Chuang TH, Hsu SC, et al. Glycolysis regulates the expansion of myeloid-derived suppressor cells in tumor-bearing hosts through prevention of ROS-mediated apoptosis. Cell Death Dis 2017;8. https://doi.org/10.1038/cddis.2017.192.

[159] Boutté AM, McDonald WH, Shyr Y, Yang L, Lin PC. Characterization of the MDSC proteome associated with metastatic murine mammary tumors using label-free mass spectrometry and shotgun proteomics. PLoS One 2011;6:e22446. https://doi.org/10.1371/journal.pone.0022446.

[160] Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, et al. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. Cell Cycle 2009;8:3984–4001. https://doi.org/10.4161/cc.8.23.10238.

[161] Pavlides S, Vera I, Gandara R, Sneddon S, Pestell RG, Mercier I, et al. Warburg meets autophagy: cancer-associated fibroblasts accelerate tumor growth and metastasis via oxidative stress, mitophagy, and aerobic glycolysis. Antioxidants Redox Signal 2012;16:1264–84. https://doi.org/10.1089/ars.2011.4243.

[162] Ueno T, Utsumi J, Toi M, Shimizu K. Characteristic gene expression profiles of human fibroblasts and breast cancer cells in a newly developed bilateral coculture system. BioMed Res Int 2015;2015:1–11. https://doi.org/10.1155/2015/960840.

[163] Migneco G, Whitaker-Menezes D, Chiavarina B, Castello-Cros R, Pavlides S, Pestell RG, et al. Glycolytic cancer associated fibroblasts promote breast cancer tumor growth, without a measurable increase in angiogenesis: evidence for stromal-epithelial metabolic coupling. Cell Cycle 2010;9:2412–22. https://doi.org/10.4161/cc.9.12.11989.

[164] Bonuccelli G, Whitaker-Menezes D, Castello-Cros R, Pavlides S, Pestell RG, Fatatis A, et al. The reverse Warburg effect: glycolysis inhibitors prevent the tumor promoting effects of caveolin-1 deficient cancer associated fibroblasts. Cell Cycle 2010;9:1960–71. https://doi.org/10.4161/cc.9.10.11601.

[165] Martinez-Outschoorn UE, Goldberg A, Lin Z, Ko YH, Flomenberg N, Wang C, et al. Anti-estrogen resistance in breast cancer is induced by the tumor microenvironment and can be overcome by inhibiting mitochondrial function in epithelial cancer cells. Cancer Biol Ther 2011;12:924–38. https://doi.org/10.4161/cbt.12.10.17780.

[166] Fiaschi T, Marini A, Giannoni E, Taddei ML, Gandellini P, De Donatis A, et al. Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay. Cancer Res 2012;72:5130–40. https://doi.org/10.1158/0008-5472.CAN-12-1949.

[167] Zhang D, Wang Y, Shi Z, Liu J, Sun P, Hou X, et al. Metabolic reprogramming of cancer-associated fibroblasts by IDH3α downregulation. Cell Rep 2015;10:1335–48. https://doi.org/10.1016/j.celrep.2015.02.006.

[168] Rattigan Y, Patel B, Ackerstaff E, Sukenick G, Koutcher A, Glod J, et al. Lactate is a mediator of metabolic cooperation between stromal carcinoma associated fibroblasts and glycolytic tumor cells in the tumor microenvironment. Exp Cell Res 2012;318:326–35. https://doi.org/10.1016/j.yexcr.2011.11.014.

[169] Patel B, Ackerstaff E, Serganova IS, Kerrigan JE, Blasberg RG, Koutcher JA, et al. Tumor stroma interaction is mediated by monocarboxylate metabolism. Exp Cell Res 2018;352:20–33. https://doi.org/10.1016/j.yexcr.2017.01.013.

[170] Lopes-Coelho F, André S, Félix A, Serpa J. Breast cancer metabolic cross-talk: fibroblasts are hubs and breast cancer cells are gatherers of lipids. Mol Cell Endocrinol 2018;462:93–106. https://doi.org/10.1016/j.mce.2017.01.031.

[171] Balaban S, Shearer RF, Lee LS, van Geldermalsen M, Schreuder M, Shtein HC, et al. Adipocyte lipolysis links obesity to breast cancer growth: adipocyte-derived fatty acids drive breast cancer cell proliferation and migration. Cancer Metabol 2017;5:1–14. https://doi.org/10.1186/s40170-016-0163-7.

[172] Wang YY, Nieto L, Gilhodes J, Milhas D, Prentki M, Guerard A, et al. Mammary adipocytes stimulate breast cancer invasion through metabolic remodeling of tumor cells. JCI Insight 2017;2. https://doi.org/10.1172/jci.insight.87489.