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Metabolic implication of tumor:stroma crosstalk in breast cancer

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Abstract The metabolic properties of cancer cells significantly differ from those of normal cells. In particular, cancer cells are largely dependent on aerobic glycolysis, a phenomenon that has been exploited clinically by using labelled glucose for positron emission tomography imaging. Importantly, cancer-associated alterations in metabolism are not merely due to the resulting response to cell proliferation and survival. Indeed, direct metabolic regulation could be driven by tumor oncogenes and/or suppressors, as demonstrated in several solid tumors, including breast cancer. Despite the fact that most breast cancer studies have focused on the intrinsic characteristics of breast tumor cells, it is now widely accepted that tumor microenvironment plays an important role in defining and reprogramming cancer cell metabolism. Tumor:stroma crosstalk, as well as inflammatory cues, concurs to outlining the cancer metabolism, impact on cancer aggressiveness and ultimately on patient survival and therapeutic responses.The aim of this review is to (i) gather the most recent data regarding the metabolic alterations in breast cancer, (ii) describe the role of tumor microenvironment in breast cancer cell metabolic reprogramming, and (iii) contemplate how targeting metabolic pathways aberrantly activated in breast cancer could help current therapeutic regimens.

Keywords Breast cancer · Tumor microenvironment · Metabolic reprogramming · Aerobic glycolysis · OXPHOS · Reverse warburg

Under physiological condition, in normal tissue, nonproliferating differentiated cells use oxidative phosphorylation (OXPHOS) for ATP production. Such cells metabolize glucose to pyruvate through glycolysis and then oxidize this pyruvate through the tricarboxylic acid (TCA) cycle generating ATP. This maximizes the efficiency of ATP production from a single molecule of glucose. Warburg original data reported that rapidly proliferating tumor cells consume glucose at a higher rate compared to normal cells and that the majority of their glucose carbon is converted to lactate, even in oxygen-rich conditions [1]. For many years, this aberrant metabolic status of cancer cells has been seen as a side effect of alterations of signal transduction pathways due to proto-oncogenes and tumor suppressors' deregulation. However, a large body of evidence is now supporting the idea that activated oncogenes and inactivated tumor suppressors directly regulate cell metabolism, hence causing tumorigenic alterations and allowing environmental change adaptation of transformed cells.

Metabolic reprogramming in cancer

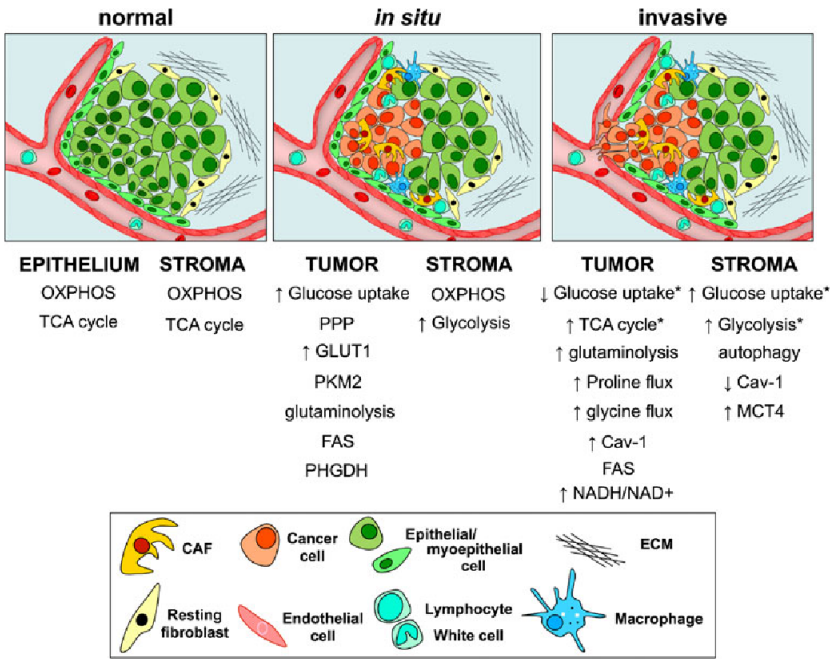
Most cancer cells, even in the presence of oxygen, show increased glycolysis followed by fermentation of the pyruvate to lactate (Warburg effect), in contrast to the conversion to acetyl CoA of pyruvate through the TCA cycle in the mitochondria that occurs in normal nonproliferating cells. The lowest yield of ATP per glucose molecule is compensated by a higher glycolytic flux that results in a higher rate of ATP production during glycolysis compared to OXPHOS [2]. In addition, glucose degradation provides cells with intermediates needed for the biosynthesis of nucleotides, lipids, amino acids, and, through the oxidative pentose phosphate pathway (PPP), NADPH for reductive biosynthetic reactions. However, mitochondria are not defective in tumor cells, as initially hypothesized by Warburg, and still have an important role particularly in the synthesis of anabolic precursors [3]. Proliferation of cancer cells not only depends on ATP production but also on biosynthesis of building blocks. To synthesize lipids, proteins, and nucleic acids, cells use glycolytic and TCA cycle intermediates. Alternatively to glucose, glutamine catabolism is an important source of biosynthetic components

in cancer cells. A wide variety of human cancer cell lines have shown sensitivity to glutamine starvation: in such "glutamine-addicted" cancer cells, mitochondria are reprogrammed to produce anabolic precursors from glutamine [4]. Despite the fact that glutamine can enter the TCA cycle becoming an important source of anabolic precursor for proliferating cells, glutamine is also a source of anaplerosis in growing cells. Thus, glutamine can be converted into glutamate; glutamate is then converted into alpha-ketoglutarate (α-KG) that enters the TCA cycle and produces oxaloacetate (OAA). In addition to its use as a source of OAA, glutamine carbon can be converted to lactate in a process that releases NADPH and NAD^+ in the cytoplasm [5]. Besides entering into the canonical TCA cycle, glutamine-derived α-KG can undergo a reductive carboxylation, catalyzed by isocitrate dehydrogenase 1 (IDH1), driving de novo lipogenesis [6, 7]. In summary, cells that efficiently convert glucose and glutamine into biomass will proliferate faster, especially in hypoxic condition [7]. The PPP is one of the main antioxidant defense mechanism for the cell. The key enzyme catalyzing the priming step of PPP is G6PD that oxidize the glucose-6-phosphate. The oxidative branch of the PPP supplies cells with NADPH, ribose-5-phosphate, and CO2. In the nonoxidative branch of PPP, ribose-5-phosphate and a second pentose derived from intermediates in a reaction catalyzed by the transketolase enzyme. In cancer cells, pentoses for DNA biosynthesis and NADPH for reactive oxygen species (ROS) scavenging are mainly provided by PPP. In fact, changes in PPP activity have been reported in cancer initiation and progression [8].

Metabolic reprogramming during cancer progression

Warburg's hypothesis that metabolic alterations are the prime cause of cancer has been gradually replaced over the last decades by the idea of cancer as a genetic disease. Consequently, altered metabolism has been downgraded as a secondary effect of the genomic mutability selected during tumor progression [9]. In the early events of carcinogenesis, tumor growth occurs in the absence of formation of new blood vessels generating a status of hypoxia and glucose shortage in the inner mass of a growing tumor. As described earlier, many tumors display higher glucose consumption through a glycolytic pathway followed by pyruvate to lactate fermentation, even in the presence of oxygen. Importantly, this glycolytic switch is not necessarily accompanied by a reduction in oxidative phosphorylation [10, 11]. Hypoxia would be an ideal candidate as a Warburg metabolism inducer. However, it is known that the glycolytic switch is acquired in the early stage of carcinogenesis even before tumors experience hypoxia. Indeed, even in normoxic conditions, many tumors use aerobic glycolysis for their metabolic requirements indicating that the Warburg effect has functions that are not solely limited to hypoxia adaptation [12]. Although Warburg's initial idea on mitochondrial impaired function in tumor cells was wrong, in the last decade, cancer research has focused on metabolism. Indeed, metabolic reprogramming is now considered a hallmark of cancer [13]. Data now support the concept that epigenetic and genetic alterations generate a metabolic phenotype that drives cancer cell growth. As a consequence, metabolic reprogramming is a primary and fundamental aspect of cell transformation [14]. Consistent with this hypothesis, activation of the tumor suppressor p53 has been shown to be critical for cell survival following glucose depletion [15]. Glucose depletion has also been shown to select tumor populations harboring specific KRAS mutations that confer the selected clones the ability to compensate glucose shortage upregulating the glucose transporter GLUT1 [16]. Alternatively, glucose deprivation induces migratory capacities toward glucose and a metabolic shift toward lactate respiration in human cancer cells [17]. In parallel to proto-oncogene activation, tumor cells can preferentially express certain isoforms of metabolic enzymes, selecting a metabolic advantageous phenotype for cancer progression. The best-described example is the pyruvate kinase, a glycolytic enzyme that converts phosphoenolpyruvate to pyruvate, with simultaneous generation of ATP. The preferential expression of the isoform M2 (PKM2) in proliferating cells suggests a protumorigenic role for this splice variant. Although recent reports have discordant data on whether PKM2 is expressed in proliferative or nonproliferative cells in tumors, it is established that PKM2 has a role in tumorigenesis [18–20]. A picture of the metabolic change that a mammary cell undergoes during cancer progression came from experiments using the immortalized non-tumorigenic MCF10 cell line and three derivative cell subclones representing different stages of carcinogenesis. Surprisingly, the percentage of glucose metabolized through glycolysis is decreased in the transformed MCF10 cells when compared to the nontransformed parental cells. The parental MCF10 cells catabolized only a minor fraction of glucose via the PPP that is conversely elevated in the more aggressive cells. Furthermore, flux through the TCA cycle is higher in the transformed cell lines. The transformed phenotype is also accompanied by increased flux through the glycine–glutamine hub and in fatty acid synthesis and oxidation. These alterations are essentially stable through additional transitions in tumor cell phenotype and are evident in metastatic cells [21]. This demonstration further supports the ability of breast cancer cells to use different types of nutrients to fuel their metabolic processes and ultimately generate the energy needed for survival and division. Notably, the genetic background affects the metabolic plasticity of cancer cells. For example, the proto-oncogene c-Myc appears to drive the selective use of glutamine as an energy source [22].

glucose-6-phosphate generate three-carbon (glyceraldehyde-3-phosphate) and seven-carbon (sedoheptulose-7-phosphate)

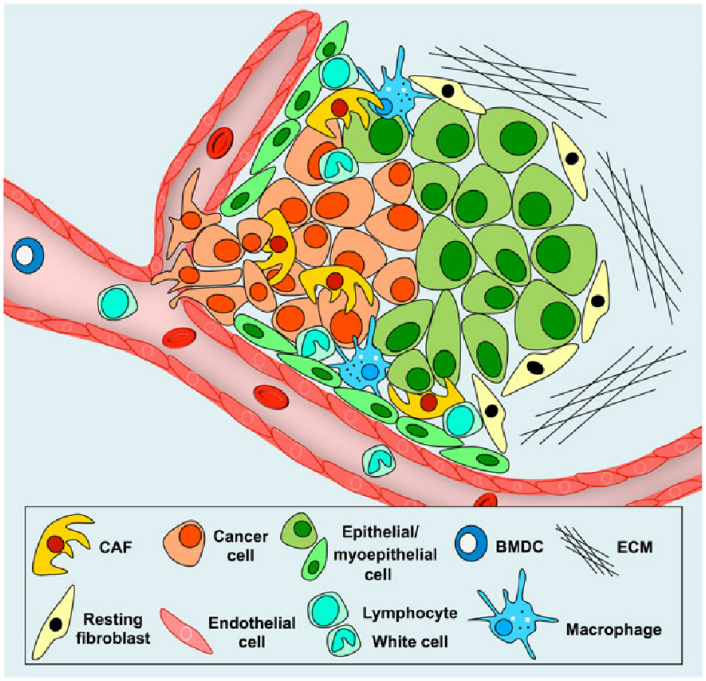


Additionally, serine biosynthesis emerged as an important metabolic pathway in the development of breast cancer. In particular, phosphoglycerate dehydrogenase (PHGDH) that catalyzes the first step of the serine biosynthesis pathway can be amplified and/or expressed at high levels in a large proportion of breast cancers and can be selected for in cancer progression (Fig. 1). In fact, breast cancers displaying high PHGDH expression have increased serine synthesis flux; the suppression of PHGDH in both in vitro and in vivo models causes a strong decrease in cell proliferation and tumor formation [23, 24]. Strong indications that altered metabolism is selected for by breast cancer cells during tumorigenesis have come from studies where combining the nuclear genome of a non-metastatic recipient cell with the mitochondrial genome of a donor aggressive breast cancer cell promoted tumor progres- sion and metastasis [25, 26]. These indications have been recently supported by the work from Felding-Habermann laboratory where enhancement of the tumor cell NAD + /NADH redox balance by mitochondrial complex I activity regulation impaired metastasis in xenograft models, increased animal survival, and strongly interfered with oncogene-driven breast cancer progression in the MMTV-PyMT mouse model [27]. Finally, oncogenic mutations of BRCA1 and BRCA2, RAS, PIK3CA and c-Myc that alter the growth and metabolism of tumor cells have been extensively reviewed elsewhere [28–30]. In parallel with these alterations that directly affect key metabolic players, breast cancer progression is characterized by upregulation of growth factor receptors that can regulate uptake of nutrients and hence affecting the energetic balance of the cell. The PI3K/AKT pathway is mutated in a high proportion of breast cancers where it regulates several critical aspects of cell growth and differentiation and is also linked to the metabolism [30]. PI3K/AKT signaling exerts a direct influence on glycolysis in cancer cells by (i) regulating the localization of the glucose transporter GLUT1 to the plasma membrane, (ii) regulating hexokinase expression, activity, and mitochondrial interaction, and (iii) indirectly activating the glycolysis rate-controlling enzyme phosphofructokinase-1 [31]. The complex nature of metabolic reprogramming of cancer cells could offer a potent therapeutic tool by inducing synthetic lethality in a subset population of cells by using a combination of drugs targeting different metabolic pathways in different stages of tumor progression.

Tumor microenvironment in breast cancer

A normal mammary duct is composed of an inner layer of luminal epithelial cells and an outer layer of myoepithelial cells surrounded by a protective basement membrane, essential for maintaining the luminal cell polarity [32]. The surrounding extracellular matrix (ECM) enables communication with the surrounding stroma. Mammary tumors arise from genetic/epigenetic changes that culminate in luminal cell proliferation, loss of epithelial polarity, and decrease of

Fig. 1 Metabolic alterations during breast tumorigenesis in cancer and stromal cells. A schematic representation of metabolic rewiring that tumor cells and stromal cells (i.e., CAFs) undergo during breast tumorigenesis. Asterisks refer to the “reverse Warburg effect” as described in the main text



myoepithelial cells as well as changes in ECM/basal membrane composition and integrity [33]. The stromal compartment of this milieu includes fibroblasts, macrophages, and leukocytes/lymphocytes as well as endothelial cells (Fig. 2). During tumor burdening and progression, the inner part of the tumor undergoes hypoxia, leading to nutrients and oxygen deprivation. This causes a shift of the cancer cells' metabolic behavior toward glycolysis inducing the acidification of the tumor microenvironment and eliciting a transcriptional response that promotes angiogenesis and local invasion [34]. During tumor progression, the numbers of cancer-associated fibroblasts (CAFs), either resident or recruited from circulating bone-marrow derived mesenchymal stem cells as well as infiltrated leukocytes are increased [35, 36]. This leads to a feed-forward loop where growth factors, cytokines, chemokines and matrix metalloproteinases (MMPs) secreted by stromal cells concur to the recruitment of macrophages, and endothelial precursor cells and regulatory lymphocytes, sustaining tumor progression [35, 37, 38] (Fig. 2). In this scenario, (i) macrophages are polarized toward the protumor/proangiogenic M2 phenotype, sustaining chronic proinflammatory signals [37, 39]; (ii) endothelial precursor cells generate new vessels [40], and (iii) lymphocytes are induced to the CD4/Treg function, causing a decreased anti-tumor immune response [41]. Noteworthy, stromal reactivity and infiltration grade as well as the ratio of macrophages/T-cells have been correlated with poor prognosis and therapy resistance in breast cancer [42].

Metabolic reprogramming of breast cancer cells is affected by tumor microenvironment

Despite a large proportion of breast cancer studies have focused on the intrinsic characteristics of tumor cells, it is now widely accepted that tumor microenvironment plays an important role in defining and reprogramming cancer cell metabolism. As described above, cancer cells are embedded within stromal cells, especially CAFs. For such a reason, it should not be surprising that CAFs can influence the metabolism of adjacent cancer cells, and vice versa. The Warburg effect could be seen as an artifact of growing cancer cells in vitro, out of their tumor context. In fact, the seminal work by Lisanti and coworkers demonstrated that in co-culture models, cancer cells induce aerobic glycolysis in stromal fibroblasts. Consequently, CAFs secrete high levels of energy-rich metabolites (e.g. lactate, pyruvate, and ketone bodies) that are used by cancer cells via OXPHOS. This phenomenon, which is demonstrated to be common to many types of cancers [43], has been termed the reverse Warburg effect [44] (Fig. 2). A similar synergetic relationship has been described for hypoxic and normoxic cancer cells; indeed,

Fig. 2 Tumor microenvironment of an infiltrating breast cancer. A normal mammary duct is composed of an inner layer of luminal epithelial cells and an outer layer of myoepithelial cells surrounded by a continuous basement membrane. Stroma containing fibroblasts, immune cells, and vasculature surrounded by the extracellular matrix maintains the normal tissue structure. In tumor burdening, altered myoepithelial cells are unable to maintain normal duct organization. CAFs and infiltrated leukocytes secrete growth factors, cytokines, chemokines, and matrix metalloproteinases to promote tumor progression. With the loss of myoepithelial cells and basement membrane, the cancer cells escape from the primary tumor site and migrate to distant organs, eventually leading to metastases

normoxic cancer cells absorb lactate produced by hypoxic cancer cells and increase OXPHOS [45]. Importantly, cancer cells may induce oxidative stress in CAFs not exclusively for their metabolic needs, but also drive their own mutagenic evolution toward a more aggressive phenotype by promoting genomic instability [46]. These data have important clinical implications; Lisanti and coworkers identified that loss of stromal caveolin-1 (Cav-1), a structural component of caveolae associated with an aggressive CAF phenotype, has independent predictive value in human breast cancer patients [47]. In addition, immunohistochemistry on a large cohort of patients that display concomitant ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) revealed a differential expression of stromal Cav-1 and monocarboxylate transporter 4 (MCT4) during the progression from DCIS to IDC. Particularly, loss of stromal Cav-1 and acquisition of MCT4 was found in the majority of the samples in the progression from in situ to invasive carcinomas. Interestingly, a concomitant loss of Cav-1 and gain of MCT4 was observed in the stroma of 75 % of the cases, when matched DCISs and IDCs were compared [48]. A loss of stromal Cav-1 in CAFs is associated with ROS production and oxidative stress. This is sufficient to induce the activation of transcription factors, such as hypoxia-inducible factor 1-alpha (HIF1α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), leading to aerobic glycolysis in CAFs even in the presence of oxygen. The activation of HIF1α and NFkB induced in adjacent fibroblasts by cancer cells eventually leads to the induction of the autophagic program [49]. During autophagy, both caveolae (marked by Cav-1) and mitochondria are destroyed by lysosomal degradation, leading to the production of highly ener- getic nutrients to support cancer cell growth; this finally promotes the onset of aerobic glycolysis in CAFs, because of their mitochondrial dysfunction. As described above, cancer cells display a high glutamine uptake, and they metabolize glutamine at a higher rate compared to other aminoacids [50]. Glutamine differently affects the tumor and stromal cells; particularly, glutamine administration in vitro (i) increases tumor cell aggressiveness by enhancing mitochondrial mass and decreasing autophagy and (ii) transforms the stroma into a tumor-promoting state, by decreasing Cav-1 expression and increasing autophagy. Therefore, glutamine-induced autophagy in fibroblasts promotes mitochondrial biogenesis in epithelial cancer cells [51]. Lisanti and coworkers propose the so-called autophagic tumor stroma model in which metabolic coupling occurs between epithelial cancer cells and the surrounding stromal cells: CAFs undergo autophagy and secrete glutamine into the tumor microenvironment. Cancer cells absorb CAF-produced glutamine that enters the TCA cycle, fuelling OXPHOS, and is concomitantly converted into ammonia. Ammonia diffuses into the microenvironment and stimulates autophagy and glutamine production in CAFs, generating a self-sustaining vicious cycle [52, 53]. The synergic relationship between tumor cells and their associated fibroblasts is therefore becoming an appealing clinical target to combat breast tumorigenesis.

Tumor microenvironment and response to therapy

Targeting the tumor microenvironment has been exploited clinically in breast cancer, and aromatase inhibitors (AIs) can be seen as tumor microenvironment-targeting therapy; AIs that target the conversion of androgens into estrogens catalyzed by aromatase, mainly expressed by stromal compo- nents, are the standard of care for postmenopausal estrogen receptor-positive breast cancer patients. Besides being a tar- get, the tumor microenvironment has a significant impact on response to therapy in breast cancer. For instance, aberrant expression of growth factor receptors [e.g., insulin-like growth factor receptor, human epidermal growth factor receptor 2 (HER2/neu) and rearranged during transfection (RET) receptor] has been shown to be responsible for AI resistance [54]. Importantly, receptor downstream signaling in epithelial cells could be triggered by stroma-produced growth factors, such as described for glial cell line-derived neurotrophic factor (GDNF)-RET [55]. Similarly, resistance to the antiestrogen fulvestrant promotes an invasive pheno- type because of an increased epithelial expression of c-MET, which is then activated by fibroblast-produced hepatocyte growth factor [56]. Furthermore, tumor microenvironment contributes to patient outcome. Stromal gene expression sig- natures have a strong prognostic value and can recapitulate the immune response as well as angiogenic and hypoxic re- sponses [57]. In addition, breast carcinomas can be divided into different subgroups with different clinical outcomes based on the expression of extracellular matrix genes, coordi- nately expressed in both neoplastic and adjacent stromal cells [58, 59]. Similarly, another stromal signature has been de- scribed to predict resistance to neoadjuvant chemotherapy in breast cancers [60]. We extensively described the metabolic implication of stromal Cav-1. In human breast cancer patients, a loss of stromal Cav-1 expression predicted tamoxifen resis- tance and poor clinical outcome [47], whereas elevated Cav-1 expression in tumor cell was associated with basal-like and metaplastic breast cancers and poor prognosis [61]. Others have reported that increased stromal Cav-1 promotes invasion and metastasis through remodeling of the stromal ECM [62]. Therefore, it becomes crucial to define whether potential markers are expressed by tumor or stromal cells for therapeu- tic targeting optimization. A major cause of breast cancer mortality is due to the metastatic spread of the disease. Although dissemination is an early event in breast cancer, relapse can occur also after decades since the first diagnosis. This recurrence is

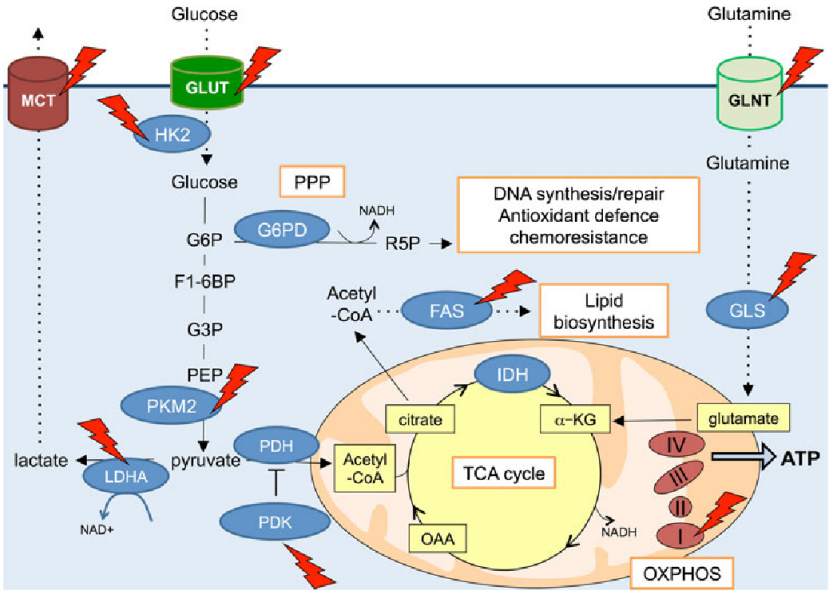


Fig. 3 Metabolic targets for cancer therapy. Several metabolic pathways of malignant cells can be targeted to inhibit tumor initiation and progression. Since the focus of the review is breast tumor, *red thunders* highlight the targets that have been reviewed and mentioned in the main text. *α-KG* α-ketoglutarate; *FAS* fatty acid synthase; *F1-6BP* fructose-1,6-bisphosphate; *G3P* glyceraldehyde 3-phosphate; *R5P* ribose 5-phosphate; *G6P* glucose-6-phosphate; *G6PD* glucose-6-phosphate dehydrogenase; *GLNT* glutamine transporter; *GLS* glutaminase; *GLUT* glucose transporter; *HK2* hexokinase 2; *I* complex I; *IDH* isocitrate dehydrogenase; *III* complex III; *IV* complex IV; *LDHA* lactate dehydrogenase A; *MCT* monocarboxylate transporter; *OAA* oxaloacetate; *PDH* pyruvate dehydrogenase complex; *PDK* pyruvate dehydrogenase kinase; *PEP* phosphoenolpyruvate; *PKM2* pyruvate kinase M2 isoform; *V* complex V

thought to be due to micrometastases that can survive in a dormant state (dormancy). Importantly, a central role for tumor microenvironment in determining whether these cells could enter or exit from dormancy has been hypothesized. A recent report demonstrates that in 3D co-culture that recapitulates the bone microenvironment of the breast cancer metastatic niche, dormant cancer cells were able to proliferate upon transfer into supportive microenvironment [63].

Exploiting metabolic reprogramming as a therapeutic target

As described above, the metabolic pathway alteration occur- ring in cancer cells has been linked to their plasticity to adapt to environmental changes and therapies. This has refocused scientists' efforts on targeting metabolic addictions of cancer cells as a selective anticancer strategy. Particularly, impairing cancer cell's energetic plasticity by targeting selective meta- bolic pathways has been proven effective to resensitize resistant cancer cells to anticancer treatments [64, 65]. The main metabolic pathways targeted by synergistic adjuvant therapeu- tic regimens are: (i) uptake/transport of key nutrients to which cancer cells are addicted, i.e., glucose, glutamine and lactate; (ii) glycolysis and the Warburg metabolism; (iii) glutaminolysis; (iv) TCA/OXPHOS and (v) fatty acid metab- olism (Fig. 3). To date, the combination of antimetabolic and chemother- apeutic regimens has produced several interesting and prom- ising results, leading to overcome resistance to standard ther- apy in several cancers [65–67]. Since the focus of our review is breast cancer, we have collected data describing the ap- proaches used for targeting glycolytic and/or respiratory path- ways in breast cancer cells. In cells that are metabolically dependent from glucose, direct targeting of GLUT1-3 transporters through specific inhibitors like phloretin has been demonstrated to sensitize tumor cells to chemotherapy to overcome drug resistance in response to hypoxia [68]. In keeping with this, it has been shown that several cancers including mammary tumors, which rely on glucose transporters to prevent apoptosis, promote cancer adaptation to hostile environment and acquire drug resistance. Patients bearing these tumors might benefit from the combinatory treatment of such inhibitors and chemother- apy [68, 69]. Besides inhibition of glucose transport, impairing glycolysis by targeting hexokinase-2 (HK) has been demonstrated to synergize with standard therapies in breast cancer [70–72]. HK, the first regulatory enzyme in glycolysis, is inhibited by a series of agents [e.g. 2-deoxyglucose (2-DG), 3-bromo pyruvate, and ionidamide] that are under clinical

investigations with ongoing clinical trials (www.clinicaltrials. gov). Several data indicate that combining 2-DG with radia- tion or chemotherapeutic treatments displays an enhanced efficacy compared to radiotherapy and chemotherapy alone. Accordingly, targeting HK synergizes with trastuzumab treat- ments in sensitive cells and overcome resistance in the resis- tant ones both in vitro and in vivo models of HER2/neu positive breast cancers [72]. Several studies showed a negative correlation between PKM2 expression and drug resistance. Although PKM2 stud- ies are considerably increased in the last years [19, 73–75] and several interesting data have been collected about the synergy between PKM2 targeting and docetaxel sensitization of colon cancer cells [76, 77], its identification as a molecular target in breast cancer is still contradictory. Additionally, cancer cells' lactate production has been targeted to inhibit acidity in tumor microenvironment and to drive pyruvate entrance into the mitochondrion. Indeed, lac- tate dehydrogenase A (LDHA) knockdown in breast cancer cells produces increased mitochondrial respiration, decreased cellular adaptation to hypoxic conditions, and impairs tumor- igenicity [78]. Furthermore, LDHA inhibition by oxomate or by FX11 [79] has been reported to resensitize breast cancer cells to paclitaxel and trastuzumab in taxol and trastuzumab resistant cells, respectively [80]. Finally, pyruvate dehydrogenase kinase (PDK) is a target of dichloroacetate (DCA), a drug under clinical investigation for its ability to induce a metabolic switch from glycolysis to mitochondrial respiration in cancer cells. Nevertheless in clin- ical trials, its effect as a single agent is limited. Conversely, DCA has been indicated as a promising drug for combinato- rial treatments for several tumors, including metastatic breast cancers [80, 81]. Lipid biosynthesis is also an attractive therapeutic target. Fatty acid synthase (FAS) has an essential functional role for growing cells; it has been indicated as a metabolic oncogene and it is overexpressed in aggressive cancers [82, 83]. Inhibi- tion of FAS by selective agents e.g., cerulenin, C75, or orlistat, has been shown to synergize with chemotherapy (docetaxel and adriamycin) and with trastuzumab in breast cancer cells [84–89]. Besides the Warburg glycolytic phenotype, the mitochon- drial respiratory behavior of cancer cells has now become interesting for oncologists. Reverse Warburg metabolism and/or engagement of TCA cycle and OXPHOS have been indicated as adaptation strategies in aggressive cancers, in- cluding mammary carcinomas [43, 90, 91]. OXPHOS impair- ment has been investigated using metformin, an antidiabetic drug in clinical use that has been also shown to inhibit com- plex I in mitochondrial electron transfer chain. Metformin anticancer activity has been reported in several cancer models, including breast cancer [92]. Besides the synergy of metformin with standard chemotherapy and its ability to reverse multidrug resistance [93], recent interesting data reveal that metformin is able to selectively target breast cancer stem cells, thereby suggesting that tumor-initiating cells are characterized by a respiratory metabolism [94]. The promising antitumoral effects of metformin and phenformin, another antidiabetic drug from the biguanide class, together with their relative low cost and low toxicity, led to several clinical trials, including a large-scale adjuvant study in breast cancer [95, 96]. Data on biguanides also support the recent findings described by Santidrian and colleagues [27] suggesting that increased NAD^+ levels, either caused by genetic or pharmacological in- terference with NAD synthesis, correlate with the im- pairment of the metastatic ability of breast cancer cells. In fact, targeting NAD synthesis with selective inhibi- tors has been proposed as a potential antineoplastic treatment [97]. Finally, in glutamine-addicted cancers, which largely de- pend on TCA cycle/OXPHOS for energy supply, glutaminolysis is also a potential pharmacological target. Shift toward the use of glutamine has been linked to drug resistance [65, 66] and is a common escaping strategy, i.e., resistance mechanism, in cancers treated with Warburg metabolism inhibitors. Inhibition of glutamine uptake through selective targeting of membrane carriers or glutaminase, the rate-limiting enzyme of glutaminolysis, has been proven success- ful in therapy. However, to our knowledge, data on breast cancer are limited [98–100]. Since glutaminolysis inhibition has been proven successful in combinatory treatment with chemotherapeutic agents, e.g., cyclophosphamide, vincristin or cysplatin, it is possible that targeting glutaminolysis in breast cancer cells may concur to synergize with standard therapeutic regimens or even revert resistant cancers to re- spond to the initial therapy.

Conclusion

As discussed in this review, the metabolic reprogramming that cancer cells undergo during tumorigenesis offers a wide range of potential targets to impair tumor initiation and progression (Fig. 3). We also described how targeting aberrant metabolic pathways in breast cancer is effective to enhance the efficacy of current therapies or even to resensitize resistant tumors. However, since fast-proliferating and neoplastic cells can display common metabolic pathways, novel delivery approaches and selective drugs should be further investigated and developed; this should induce synthetic lethality in tumor cells and the therapeutic regimen be tolerable for the patients. Finally, understanding the metabolic reprogramming during tumorigenesis and therapy response is pivotal to maximize the efficacy of current antineoplastic treatment and ameliorate breast cancer patients' prognosis.

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