Metabolism within the tumor microenvironment and its implication on cancer progression: an ongoing therapeutic target

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ABSTRACT

Since reprogramming energy metabolism is considered a new hallmark of cancer, tumor metabolism is again in the spotlight of cancer research. Many studies have been carried out and many possible therapies have been developed in the last years. However, tumor cells are not alone. A series of extracellular components and stromal cells, such as endothelial cells, cancer-associated fibroblasts, tumor-associated macrophages and tumor-infiltrating T cells, surround tumor cells in the so-called tumor microenvironment. Metabolic features of these cells are being studied in deep in order to find relationships between metabolism within the tumor microenvironment and tumor progression. Moreover, it cannot be forgotten that tumor growth is able to modulate host metabolism and homeostasis, so that tumor microenvironment is not the whole story. Importantly, the metabolic switch in cancer is just a consequence of the flexibility and adaptability of metabolism and should not be surprising. Treatments of cancer patients with combined therapies including anti-tumor agents with those targeting stromal cell metabolism, anti-angiogenic drugs and/or immunotherapy are being developed as promising therapeutics.

Keywords

Metabolism; tumor microenvironment; endothelial cells; immune cells; stromal cells; angiogenesis; immunosuppression

Abbreviations: Arg1, arginase 1; ASNS, asparagine synthetase; bFGF, basic fibroblast growth factor; CAFs, cancer-associated fibroblasts; CPT1, carnitine palmitoyltransferase 1; ECs, endothelial cells; ECM, extracellular matrix; FAO, fatty acid oxidation; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; GLS, glutaminase; GS, glutamine synthetase; HBP, hexosamine biosynthesis pathway; HIF-1α, hypoxia inducible factor 1α; HK, hexokinase; IDO, indoleamine-2,3-dioxygenase; iNOS, inducible nitric oxide synthase; LDH, lactate dehydrogenase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; NO, nitric oxide; ODC, ornithine decarboxylase; OXPHOS, oxidative phosphorylation; PCK1, phosphoenolpyruvate carboxykinase 1; PD-1, programmed death 1 receptor; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; PFK1, 6-phosphofructokinase; PFKFB3, phosphofructokinase-2/fructose-2,6-bisphosphatase 3; PHD, prolyl hydroxylase; PK, pyruvate kinase; PPP, pentose phosphate pathway; ROS, reactive oxygen species; TAMs, tumor-associated macrophages; TCA, tricarboxylic acid cycle; TDO, tryptophan-2,3-dioxygenase; TILs, tumor-infiltrating lymphocytes; TME, tumor microenvironment; uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.
1. INTRODUCTION

Otto Warburg started studying tumor metabolism in the first years of the 20th century and 30 years later it was proposed what we now call the “Warburg effect”.1,2 During the next years, cancer metabolism was an emerging issue in biological research, although there was a fall of interest for some years because of the boom of Molecular Biology, thought to be able to give meaningful answers to almost all questions.3 However, many studies have been performed in the last decade due to a renewed interest in tumor metabolism, so that nowadays reprogramming energy metabolism has been considered a new hallmark of cancer.4 By means of both classical and modern techniques, many new relevant features of metabolism of cancer cells have been discovered. Moreover, tumor cells are not alone, since a complete set of stromal and immune cells meet in the so called “tumor microenvironment” (TME), along with extracellular matrix (ECM), which provides more than an inert playground for this game.5 These cells include endothelial cells (ECs) (vascular or lymphatic) and associated pericytes, cancer-associated fibroblasts (CAFs), and immune cells, such as tumor-infiltrating lymphocytes (TILs) (T cells, B cells and NK cells), tumor-associated macrophages (TAMs) and mast cells.6 Studies have been usually focused on tumor and ECs metabolism. However, in the last years the metabolism of immune cells, mainly macrophages and T cells, has attracted the interest of scientific community, along with that of CAFs, due to their contribution to tumor growth. However, little is known about mast cells metabolism, and that of pericytes still remains a mystery.

Increasing knowledge about metabolism of cells of the TME will allow for the design of new therapies for cancer patients. Many compounds have already been tested for the inhibition of tumor cell metabolism, either aerobic glycolysis, glutaminolysis or other metabolic targets.7-13 New approaches for therapy are also being developed using metabolism of stromal and immune cells as a target.14-17

There are many published works about metabolism of stromal and immune cells in the TME and their relationship with tumor progression. A recent review collected the effects of tumor metabolism in the TME.18 Nevertheless, to our knowledge the relation between metabolism of the different cells of the microenvironment and tumor progression has not been well documented in a single review so far (see Supplementary Table 1). This review will try to shed light on the remarkable metabolic features of different cells of the TME and their relation with tumor progression, as well as proposing feasible therapies based on possible metabolic targets that would help in the inhibition of tumor growth and metastasis.

2. TUMOR CELLS METABOLISM: BEYOND WARBURG EFFECT

The experiments carried out by Otto Warburg in the mid-twenties of the 20th century were just the beginning of an advanced knowledge in cancer metabolism. As early as
1925, he observed a huge amount of lactic acid in rat carcinoma even when oxygen was available, a process known as aerobic glycolysis. This contradicted the well-established Pasteur effect, based on the inhibition of glycolysis in the presence of oxygen. Warburg also observed that malignant tumors produced more lactic acid than benign tumors. Nowadays we know that the glycolytic rate can be a sign of tumor aggressiveness. For example, the non-invasive MCF7 breast cancer cell line has a lower rate of aerobic glycolysis than the highly invasive MDA-MB-231 breast cancer cell line, corresponding to lower levels of lactate dehydrogenase-A (LDH-A) and to the oxidative source of the great majority of the ATP produced by MCF-7 cells. However, aerobic glycolysis is not just a sign of tumor aggressiveness, since some proliferating non-transformed cells show this metabolic characteristic too. 30 years after initial Warburg’s seminal observations, when many metabolic routes had been already discovered, he noticed that cancer cells could obtain similar amounts of energy by aerobic glycolysis and by oxidative phosphorylation (OXPHOS), in spite of the lower efficiency in ATP yielded per molecule of glucose provided by glycolysis. At that moment, it was difficult to find an explanation for this fact, since high rates of tumor cell proliferation would require the production of great amounts of energy in the form of ATP molecules, and OXPHOS was the obvious road to fulfill this purpose. Now we know that, due to that high proliferation, cancer cells have a large demand of the precursors for the new daughter cells generated by mitosis, in form of nucleotides, amino acids and lipids. Thus, glucose would be diverted to the formation of acetyl-CoA for fatty acid synthesis, glycolytic intermediates for non-essential amino acids, and ribose for nucleotides. This explains why many types of cancer cells switch their glucose metabolism towards aerobic glycolysis. Extracellular flux analyzers are currently very popular tools for measuring basic metabolism, since they are able to estimate OXPHOS through oxygen consumption rate (OCR) and aerobic glycolysis through extracellular acidification rate (ECAR). Nevertheless, studies with isolated tumors from mice showed that although progressive tumors have higher ECAR levels than regressive ones, their proliferation rates are similar, demonstrating that proliferation is not the only reason for aerobic glycolysis in tumor cells.

The increased glucose consumption by many cancers is the basis for the use of the glucose analogue 2-[\textsuperscript{18}F]-fluoro-2-deoxy-D-glucose for tumor diagnostic and treatment follow-up by using positron emission tomography (PET). In high contrast with the affirmation that all tumor cells rely mostly on aerobic glycolysis, there is ample evidence that not all cancer cells obey this rule. For example, glutamine is the major energy source for cervix adenocarcinoma HeLa cells, and Gentric et al. have reported some examples of oxidative tumors. Furthermore, oxidative and glycolytic cancer cells can co-exist within the same tumor, and a lactate shuttle is established between both of them. Lactate uptaken by oxidative cancer cells (either from other cells or from the circulation) can provide carbon skeletons to be incorporated to the tricarboxylic acid cycle (TCA) in order to obtain energy. We would like to emphasize that in the next sections and figures of this review we will not make a distinction between oxidative and glycolytic tumor cells for the sake of simplicity. It should be
taken into account that different metabolic events here represented in the same tumor cell might be occurring in different cancer cells, though.

Nonetheless, other substrates different to glucose are also differentially consumed by tumors as well. In particular, glutamine, the most abundant circulating amino acid in blood, has a major role regarding tumor growth, as glucose can only provide carbon skeletons for scaffolds of new molecules and glutamine would serve as a nitrogen source.\(^{31}\) In fact, glutamine is a non-essential amino acid for non-transformed human cells but it turns into an essential amino acid for tumor cells.\(^{12}\) Moreover, a host to tumor net flux of glutamine has been confirmed in mice inoculated with Ehrlich ascites tumor cells, enabled by an increased contribution made by the host tissues to circulating glutamine during tumor development.\(^{32,33}\) We will discuss this issue in a later section of this review.

Almost 30 years ago, our group found out that Ehrlich ascites tumor cells, grown under steady state conditions, utilize both glucose and glutamine, producing two moles of lactate per mole of glucose, and one of glutamate and ammonia per mole of glutamine consumed.\(^{34}\) That means that cancer cells are able to use glucose and glutamine in a completely dissipative way. Both nutrients are important, as they lead to ATP production and provide intermediates for macromolecular synthesis. The roles of glutamine in intermediary metabolism have already been revised.\(^{35}\) Additionally, glutamine can be used for synthetizing the non-essential amino acids alanine, serine, arginine and proline and also fatty acids, although glucose is the major lipogenic substrate, as seen in glioblastoma cells.\(^{36,37}\) It is important to remember that glutamine can lead to lactate production through glutaminolysis. So, aerobic glycolysis is not the only way a tumor cell possesses to produce lactate, whose excretion out of the cell was first thought to be a mechanism to eliminate the pyruvate excess.\(^{23}\) However, lactate would have many roles in benefit of tumor progression that will be discussed in other sections of the present review. Likewise, ammonia was also thought to be just a toxic waste product. Nevertheless, it has been recently shown that this metabolite can be recycled to generate amino acids through glutamate dehydrogenase (GDH) activity, providing a nitrogen source to the tumor.\(^{38}\)

Metabolic profiling depends on cell distribution, as cancer cells within the oxygenated periphery may consume and oxidize the lactate resulting from aerobic glycolysis by cells in the hypoxic area.\(^{39}\) Besides, cancer metabolic phenotypes are usually defined by the origin of the tissue, epigenetic drivers, aberrant signaling, and the TME.\(^{40}\) Indeed, genetics, epigenetics and metabolism interact with one another and, as a result, tumor heterogeneity is the overall result of all these changes at different levels.\(^{41}\) A previous review of tumor metabolism contributed by our group focused its attention in the genetic regulation of tumor metabolism. The key roles played by c-myc, K-Ras and p53 are well documented. For example, c-myc oncogene promotes expression of LDH-A, the glutamine transporter SLC1A5 and GLS glutaminase (associated to tumor malignancy), and K-Ras stimulates glucose uptake, lactate production and canalization.
of glutamine carbons to the Krebs cycle, whereas tumor suppressor gene p53 induces GLS2 glutaminase expression (typical of non-proliferative cells), OXPHOS and fatty acid oxidation (FAO), and diminishes expression of glucose transporters and some of the key glycolytic enzymes.\textsuperscript{42}

Epigenetics plays also a role in tumor metabolism. For example, 2-hydroxyglutarate (2-HG), a product of the reaction catalyzed by a mutated isocitrate dehydrogenase 1 (IDH1), inhibits the binding of α-ketoglutarate (α-KG) to tet methylcytosine dioxygenase 2 (TET2) and lysine demethylase 3A (KDM3A), two epigenetic enzymes, impairing their function.\textsuperscript{43} Another example is nitric oxide (NO), also able to drive epigenetic modifications related with tumorigenesis.\textsuperscript{44}

Less attention has been paid to studying the role of fatty acids in tumor growth, since glucose and glutamine are considered the major sources of energy in these cells. A relationship between glycolysis and FAO has been found in tumors, since highly glycolytic cell lines present a low lipid oxidation and \textit{vice versa}.\textsuperscript{45,46} Some tumors lack carnitine palmitoyltransferase 1a (CPT1a) activity, a rate-limiting enzyme of FAO.\textsuperscript{47} In various tumor cell lines, rates of oxidation of glucose higher than those of palmitate have been documented.\textsuperscript{48} However, it has been shown that highly proliferative cancer cells have a strong lipid avidity, increasing the uptake of exogenous lipids or promoting lipogenesis and cholesterol synthesis.\textsuperscript{49} Fatty acid synthase (FAS) is overexpressed in several types of cancer.\textsuperscript{50–52} Transcription factors SREBP1 and SREBP2, involved in fatty acid and cholesterol biosynthesis, are also overexpressed in many tumors.\textsuperscript{53} On the other hand, prostate tumors display a low rate of glucose utilization; they rather have a high rate of fatty acids uptake and overexpress some β-oxidation enzymes.\textsuperscript{53} It has been shown that leukemia cells require this metabolic route for proliferation and survival.\textsuperscript{54} Additionally, there is some controversy about the role of fatty acids on metastasis and invasiveness. A published study found an inverse relationship between expression of CD36, a known transporter of long fatty acids, and the metastatic potential of tumors, whereas the authors of a more recent paper postulate a positive role of CD36 in metastasis.\textsuperscript{55,56}

Other metabolites could also play essential roles in tumor metabolism. The role of asparagine in cell survival has been well-known for many years, and several studies are being carried out nowadays regarding the importance of this amino acid. The presence of asparagine is essential for maintaining cell viability in glutamine-depletion conditions, and inhibition of asparagine synthetase (ASNS), an enzyme that catalyzes the conversion of aspartate and glutamine into asparagine, leads to cell death even in a glutamine-rich media.\textsuperscript{57} Therefore, depleting asparagine and inhibiting ASNS expression seems to be a way to stop tumor growth. Treatment with the enzyme asparaginase, which is able to undermine asparagine levels in the media, has been carried out in leukemia and lymphomas since the discovery of its anti-cancer effect in 1963.\textsuperscript{58} Later, it would be known that asparaginase treatment was effective due to the null or low expression of ASNS in these tumors.\textsuperscript{59,60} Nevertheless, most solid tumors
present ASNS expression and therefore depletion of glutamine is also important for asparaginase-dependent therapy in ASNS-expressing tumors.\textsuperscript{61,62} Indeed, a study determined that glioblastoma cells that are not sensitive to glutamine deprivation are also insensitive to asparaginase treatment, but the treatment affected glioblastoma cells sensitive to deprivation of this amino acid.\textsuperscript{63} This may be due to the fact that most asparaginases also present glutaminase activity.

There are other amino acids that are essential for tumor growth and progression as well. Serine can be synthetized from glycolytic intermediates and later converted into glycine. Both amino acids are necessary for protein, nucleic acid and lipid synthesis. Serine can contribute to the formation of other metabolites by anaplerosis, being necessary for proliferation. Glycine, which may also derive from threonine, is related to folate metabolism (essential for tumor progression), to DNA methylation, and to the redox balance maintenance.\textsuperscript{64,65} Indeed, expression of PHGDH (phosphoglycerate dehydrogenase), the first enzyme in serine synthesis, is normally upregulated in triple-negative breast cancer, evidencing the importance of this amino acid for these tumors.\textsuperscript{66} In contrast, metabolism of other amino acids can be toxic for tumor cells. For example, proline oxidase (PRODH), the first enzyme in the catabolism of proline, is induced by p53.\textsuperscript{67} Expression of PRODH leads to cell cycle arrest and apoptosis in tumors, and it has been seen that c-myc inhibits its function.\textsuperscript{68}

In addition to all this, other metabolites are also important for tumors. NO is the product of the enzymatic reaction catalyzed by nitric oxide synthase (NOS), which uses arginine as substrate, as well as NADPH. Thus, the pentose phosphate pathway (PPP) would provide the reducing agent necessary for synthetizing NO. In hypoxic tumors, hypoxia inducible factor 1α (HIF-1α) interacts with IFN-γ thus inducing the expression of inducible NOS (iNOS).\textsuperscript{69} NO produced and secreted by tumor cells reprograms stromal cells to support tumor progression, although high concentrations has been shown to induce apoptosis, and it also helps drug resistance and migration of cancer cells.\textsuperscript{70-72} Moreover, NO modulates metabolism of tumor cells, inhibiting prolyl hydroxylase 2 (PHD2) and OXPHOS, hence promoting a glycolytic metabolism.\textsuperscript{73,74} Furthermore, S-nitrosylation is a mechanism of posttranslational protein modification mediated by NO and implied in modulating the activity of several oncogenic signaling cascades and metabolic enzymes.\textsuperscript{69}

Last but not least, polyamine synthesis has been known to be essential for tumor progression since the late sixties.\textsuperscript{75} High levels of intracellular polyamines have been shown to increase cell proliferation, decrease apoptosis, enhance expression of genes affecting tumor invasion and metastasis, and they are also related to angiogenesis.\textsuperscript{76} The synthesis of these macromolecules requires conversion of arginine to ornithine through arginase activity. Then, ornithine is decarboxylated to produce putrescine, the first polyamine, in a reaction catalyzed by ornithine decarboxylase (ODC), and spermidine and spermine are synthetized using decarboxylated S-adenosylmethionine (dcSAM) as an aminopropyl group donor.\textsuperscript{77} ODC was described as a proto-oncogene as soon as
1992, and ODC levels are higher in tumors than in non-proliferating tissues.\textsuperscript{78,79} Moreover, several oncogenes, such as myc and K-Ras, are responsible for augmented polyamine synthesis and decreased polyamine catabolism, thus promoting tumor progression.\textsuperscript{80–82} Interestingly, NO is able to inhibit ODC by nitrosylation.\textsuperscript{83} Polyamine synthesis in tumors has been classically suppressed by treatment with difluoromethylornithine (DFMO), an inhibitor of ODC.\textsuperscript{84} Recent research has found that mammalian target of rapamycin complex 1 (mTORC1) sustains polyamine synthesis in tumors through overexpression of S-adenosylmethionine decarboxylase 1 (AMD1), the enzyme responsible for SAM decarboxylation.\textsuperscript{85}

The different metabolic features of tumor cells mentioned here are collected in Figure 1. Taking into account all this information, it cannot be said that all tumor cells rely just on aerobic glycolysis for its growth and progression. In fact, this depends more on the kind and stage of the tumor, as well as on its microenvironment. Metabolism of different cells of this TME will be presented throughout this review, along with a recapitulation of the feasible reasons and/or consequences of those metabolic features in cancer disease.

3. METABOLISM OF CELLS AT THE TUMOR MICROENVIRONMENT

3.1. Endothelial cells

ECs are the most studied stromal cells in the TME, since they are responsible for the angiogenic process. Angiogenesis is the formation of new blood vessels from the pre-existing vascular bed. Pathological activation of angiogenesis in tumors (a process called tumor angiogenesis) allows them to grow and metastatize. This angiogenic switch is controlled by pro- and anti-angiogenic molecules secreted from different cells of the TME.\textsuperscript{86} As we discuss throughout this review, metabolic pathways regulate some of these angiogenic molecules, representing promising targets to modulate tumor angiogenesis. Therefore, targeting metabolism to inhibit tumor proliferation could be also a way to modulate the angiogenic process.

Regarding EC metabolism, there are some discrepancies among published data. Back in 1991, Spolarics et al. determined that rat liver ECs rely predominantly on aerobic metabolism rather than glycolysis, with 45% of total ATP produced by oxidation of palmitate, and 26% derived from glutamine.\textsuperscript{87} Three years before, Leighton and colleagues measured glutaminase activity in bovine pulmonary ECs, and found that it was almost 20-fold higher in comparison with that of rat lymphocytes, giving a major importance to glutamine metabolism in these cells. They also recognized some relevance to FAO, since CPT1a showed an elevated expression. However, in contrast with the results from Spolarics's group, their data showed high activity of some key glycolytic enzymes, such as hexokinase (HK), 6-phosphofructokinase (PFK1) and
pyruvate kinase (PK), suggesting that glycolysis could play an important role in EC metabolism. Indeed, other groups found glycolysis to be predominant in bovine cavernous, rat coronary and human umbilical vascular ECs (HUVEC) even in the presence of oxygen. From these and other data, it has been proposed that ECs rely on glutamine and fatty acid metabolism when the supply of glucose decreases. Most of these differences observed in bibliography could be probably due to different isolation and culture conditions of ECs, affecting their proliferation rate and their metabolism.

The interest on EC metabolism was pushed into background for some years, until 2013, when Peter Carmeliet’s laboratory found interesting data regarding the role of phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB3) activity in EC metabolism and angiogenesis. In their experiments, they observed that ECs isolated from several tissues were highly glycolytic, >200 fold-higher compared to oxidation of glucose, glutamine or fatty acids in the same cells, generating up to 85% of the total cellular ATP content only through this pathway. In addition, a reported low OCR in HUVEC may indicate that they rely more on glycolysis than on OXPHOS. These observations agree with previous results from other groups and disagree with other available data, as seen above.

PPP is also important for ECs, since it leads to the formation of reduction equivalents as NADPH, induces the synthesis of NO, a pro-angiogenic factor, and prevents the formation of reactive oxygen species (ROS). Indeed, studies in ECs have shown that an overexpression of the limiting enzyme of the PPP, glucose-6-phosphate dehydrogenase (G6PDH), results in an increase of NO synthesis, whereas its downregulation drives to an elevation in ROS levels. On the other hand, a part of the glucose metabolic flux is derived to the hexosamine biosynthesis pathway (HBP), essential for the N-linked glycosylation process. HBP may play a role in angiogenesis switch on, since VEGFR2, the key vascular endothelial growth factor (VEGF) receptor involved in tumor angiogenesis, has to be N-glycosylated to become fully functional. Regarding tumor progression, glycolysis-derived lactate has also an important role on the angiogenesis process (see section 4.2 below).

In spite of the rediscovered importance on endothelial glycolysis, glutamine metabolism is still considered to have an essential role in EC survival, as well as in angiogenesis. However, glutamine helps EC proliferation but not migration. A part of the importance of glutamine metabolism in EC survival and angiogenesis could be due to the role of this amino acid in the synthesis of polyamines, considered to be essential to EC proliferation and angiogenesis, as well as for cell survival. In fact, in some EC lines about a 26% of ornithine, the precursor for polyamine synthesis, is formed from glutamine. In addition, glutamine is also essential for asparagine synthesis through ASNS activity, as seen above. A recent study showed that asparagine can be uptaken from the media or synthetized by ASNS in ECs, and this amino acid has
an important role in protein synthesis, mTOR activation and endoplasmic reticulum (ER) stress suppression due to glutamine deprivation.\textsuperscript{99}

As mentioned above, Spolarics et al. suggested that fatty acids could be important fuels for ECs, in high contrast with previous observations from other group.\textsuperscript{87,105} A recent study from the same group that underestimated the use of fatty acids in ECs, showed later that FAO is essential for angiogenesis by promoting the \textit{de novo} synthesis of nucleotides, thus allowing ECs to proliferate.\textsuperscript{93,106} In fact, inhibition of CPT1a impaired angiogenesis in HUVEC.\textsuperscript{106} One of the long chain fatty acids transporters in ECs is CD36. Inhibition of CD36 has been shown to reduce angiogenesis, but it is not clear whether this effect is due to fatty acid uptake inhibition or not.\textsuperscript{107}

Metabolism of ECs is summarized in Fig. 1. For additional information, we encourage our readers to visit some recent reviews on EC metabolism summarizing what is known about glucose, glutamine and fatty acid fate in these cells.\textsuperscript{107–110}

### 3.2. Cancer-associated fibroblasts

CAFs are the most abundant cells within tumor stroma. They are recruited by tumor cell-secreted platelet-derived growth factor (PDGF).\textsuperscript{111} It is well known that CAFs promote tumor growth and invasion, although recently published works showed contradictory results regarding intestinal tumorigenesis.\textsuperscript{112–114}

Although from now on we will assume the classical view, it should be clear that metabolism and signaling pathways are complex and probably there is not an absolute truth. Bearing this in mind, it has been shown that CAFs resemble myofibroblasts, as they express smooth muscle cell markers and produce transforming growth factor β (TGF-β) and stromal cell-derived factor 1 (SDF1). Additionally, CAFs express the migration stimulating factor (MSF), whose overexpression leads to Akt pathway activation, which in turn induces the mTOR signaling pathway.\textsuperscript{115} CAFs expressing MSF showed elevated lactate secretion.\textsuperscript{115} Since mTOR is known to enhance glycolysis, it could be proposed that MSF increases the glycolysis rate in CAFs through mTOR signaling. This high lactate secretion by CAFs is supported by the upregulation of MCT4, a lactate exporter, observed in these cells.\textsuperscript{116} Zhang and colleagues demonstrated that IDH3α, a TCA enzyme, is downregulated in CAFs, and this situation leads to HIF-1α stabilization, resulting in a switch from OXPHOS to glycolysis.\textsuperscript{117} As we will see below, tumor cells could as well induce this glycolysis activation. Moreover, CAFs are also able to take up lactate (secreted by tumor cells) through MCT1, a lactate importer, and to oxidize it.\textsuperscript{118,119}

It has been shown that CAFs have a metabolic activity higher than that of other fibroblasts, since they present higher expression levels of glutamine synthetase (GS), of several glycolysis, TCA cycle and ETC gene products, and aspartate and asparagine
(both required for glutamine synthesis in these cells) transporters. A summary of CAFs metabolism is presented in Fig. 1. The importance of glutamine and fatty acid synthesis by CAFs in the TME will be discussed later.

3.3. Tumor-associated macrophages

Macrophages are a population of immune cells originated from bone marrow-derived monocytes (BMDM) and exhibiting a great heterogeneity in phenotype and functions. These cells help tumors to grow and invade other tissues, promoting tumor progression also by stimulating angiogenesis and inhibiting the immune response. As in the case of CAFs, the energetic metabolism of non-tumoral macrophages has been more studied than that of TAMs.

According to the activation pathway, there are two main subtypes of macrophages: M1 macrophages, activated by the canonical pathway in response to IFN-γ and LPS stimulation, and M2 macrophages, activated by an alternative pathway in response to interleukins IL-4, IL-10 and IL-13. M1 macrophages secrete pro-inflammatory cytokines and have an anti-tumoral activity, while M2 macrophages have anti-inflammatory properties. Some authors maintain that TAMs share many, but not all, features of M2 phenotype, whereas others did not find M2 markers in TAMs. However, IL-4 is sufficient for TAM polarization after monocyte recruitment by cytokines such as CCL2 and CSF-1. Moreover, a transcriptome study determined that TAMs shared genes with both M1 and M2 macrophages.

It is well-established that M1 macrophages rely largely on aerobic glycolysis, maybe regulated by itaconate. M2 macrophages have not remarkable glucose consumption rates. In contrast, high FAO and OXPHOS have been found in these cells. On the other hand, M1 macrophages were found to have enhanced expression of PFKFB3 isoenzyme, whereas alternatively-activated macrophages express it at low rates. Since PFKFB3 is a signal of high glycolytic rates, as happened in ECs, it can be said that M2 macrophage energy metabolism does not rely on this route. Another finding suggests that succinate could be a possible indirect modulator of glycolysis. Succinate is able to inhibit PHD, leading to an increased HIF-1α stabilization, as seen before in other types of cells. This high stabilization of HIF-1α might have two major consequences at the transcriptional level: i) HIF-1α can be translocated into the nucleus, together with the glycolytic enzyme PKM2. In the nucleus, HIF-1α forms a complex with HIF-1β and other regulatory proteins, thus acting as a transcription factor able to activate the expression of key glycolytic enzymes, such as glucose transporter GLUT-1, pyruvate dehydrogenase kinase-1 (PDK1) and LDH-A. ii) The same transcription factor complex can bind to the pro-inflammatory cytokine IL-1β promoter gene and activate its transcription too. In summary, succinate would have a role enhancing aerobic glycolysis and the Warburg effect, and promoting IL-1β production. Both characteristics are typical features of classically-activated macrophages. Succinate may
proceed from the anaplerotic use of glutamine, or be accumulated due to a truncated TCA cycle. Since M2 macrophages obtain energy mainly by means of FAO andOXPHOS, they do not increase succinate levels and the glycolytic pathway is notenhanced in these cells. HIF-1α can also be activated through the mTOR signalingpathway. Cytokines IL-4 and IL-13, responsible for the alternative activation ofmacrophages, inhibit mTOR via activation of the negative regulators TSC1 andTSC2. Therefore, M2 macrophages are predisposed to oxidative metabolism throughaglycolysis inhibition via mTOR/HIF-1α inactivation.

Since M1 macrophages have an anti-tumoral activity, it should be expected thatTAMs have a metabolic profile more similar to that of M2 macrophages. However,recent evidence reveals a high glycolytic rate in TAMs. Moreover, an elevatedeicosanoid production has been found in these cells and, on the other hand, inhibitionof β-oxidation did not affect cytokine production in thyroid cancer-inducedmacrophages, showing the importance of FA synthesis rather than catabolism inTAMs. Regarding amino acid metabolism, TAMs from glioblastoma or exposed toglioblastoma cells present an enhanced expression of genes related to glutamatetransport and metabolism (Fig. 1).

Serine has been shown to be an allosteric activator of PKM2. Therefore, it couldseem unlikely that M2 macrophages depend on serine utilization because theirmetabolism does not rely on an enhanced aerobic glycolysis. However, serine metabolism has been reported as an enriched pathway in M2 macrophages by usingLC/MS-based metabolomics. These last authors also found that Akt/mTORC1pathway plays a role in increasing glucose metabolism in M2 macrophages as seen byboth elevated OCR and ECAR. Therefore, there are some contradictory results fromdifferent groups. However, to our knowledge there is not available data about serine metabolism in TAMs. It would be interesting to further investigate the metabolicphenotype of these cells as well as the signaling pathways that govern them.

3.4. Tumor-infiltrating lymphocytes

T cells represent the most abundant lymphocyte population involved in the adaptiveimmune system. There are two major types of T cells: CD4+ and CD8+, which areclassified into different subtypes. CD8+ T cells often differentiate into cytotoxic T cells(CTLs), characterized by inducing apoptosis in targeted cells. CD4+ naïve T cells canbecome regulatory or suppressor T cells (Treg cells), which have immunosuppressivefunctions, or helper T cells (Th cells), a type of effector T cells that participate in theimmune response. There are many subtypes of Th cells, including pro-inflammatory(Th1 and Th17 cells) and anti-inflammatory (Th2 cells) lymphocytes, according to thecytokines secreted by them. Therefore, effector T cells include CTLs and Th cells. Mostof tumor-infiltrating lymphocytes (TILs) are Treg cells.
There is clear evidence that activation of T cells requires a metabolic switch similar to that undergone by many tumor cells, thus exhibiting the Warburg effect and an elevated aerobic glycolysis.\textsuperscript{141} Something similar happens in the innate immune system.\textsuperscript{142} Nevertheless, this metabolic switch in T cells and tumor cells is based on different causes: for T cells, this is a physiological adaptation process, whereas for tumor cells it depends on a series of intrinsic genetic mutations and external responses to the TME.\textsuperscript{143} On the other hand, Treg and memory CD\textsuperscript{8}\textsuperscript{+} T cells rely on FAO and OXPHOS for its survival and differentiation. Additionally, it has been reported that \textit{de novo} lipogenesis is required for Treg differentiation from Th17 lymphocytes (Fig. 1).\textsuperscript{144} Effector T cells, nonetheless, can survive utilizing OXPHOS in case of glucose depletion, although cytokine production is diminished under these conditions.\textsuperscript{145}

Phosphoenolpyruvate (PEP) has been related to the T cell receptor (TCR) activation through Ca\textsuperscript{2+} flux. Ho et al. observed that overexpression of phosphoenolpyruvate carboxykinase 1 (PCK1), the enzyme that catalyzes the conversion of oxaloacetate into PEP, restored PEP levels and Ca\textsuperscript{2+} flux in glucose-deprived T cells. This can be explained by the fact that PEP undermines the activity of SERCA, an ER calcium ATPase. Under these conditions, Ca\textsuperscript{2+} escapes from ER to cytosol, increasing TCR-induced Ca\textsuperscript{2+} flux and effector function. Moreover, TCR is able to activate glucose metabolism enhancing PKM2 activity, which in turn could contribute to PEP accumulation.\textsuperscript{146} Thus, T cell effector function would be partially controlled by PCK1 activity.

As for other cell types, mTOR plays a crucial role in T cell metabolism. Inhibition of mTOR results in an induction of AMPK phosphorylation and, consequently, an increase of FAO, leading to differentiation of CD\textsuperscript{4}\textsuperscript{+} T cells to Treg. Thus, mTOR would guide these cells to Th1, Th2 and Th17 phenotypes.\textsuperscript{147,148} Programmed death 1 receptor (PD-1), an inhibitory checkpoint receptor present in TILs, has an important role in regulating glycolysis through mTOR signaling pathways. This issue will be clarified in sections below.

Dang et al. demonstrated that HIF-1\textalpha is able to induce Th17 differentiation through transcriptional activation of RORγt. HIF-1\textalpha also binds to Foxp3, targeting it for its degradation and impairing this molecule to promote Treg development.\textsuperscript{149} Therefore, HIF-1\textalpha would promote a glycolytic cell phenotype (by activating Th17 cells) while inhibiting oxidative metabolism (via Treg impairment).

However, glycolysis is not the only pathway necessary for T cell activation. c-Myc-dependent glutaminolysis is also essential for proper T cell effector function, as it leads to nucleotide and polyamine synthesis, necessary for supporting cell proliferation.\textsuperscript{150} In addition, glutamine regulates T cell proliferation as well as it increases IL-2 production and IL-2 receptor expression.\textsuperscript{151} Arginine has also been shown to improve survival and anti-tumor activity of T cells.\textsuperscript{152} An overview of TILs metabolism is presented in Fig. 1.
3.5 The tumor microenvironment forgotten cells

There are many different kinds of cells in the TME, and the ones presented here up to now are just the more abundant and studied. Tumor-associated mast cells (TAMCs) and tumor-associated pericytes are also predominant cells in the TME and have an important role in tumor progression. However, their metabolism, as far as we know, have not been described to date.

3.5.1. Tumor-associated mast cells

TAMCs are recruited to tumors in response to stem cell factor (SCF) from tumor cells and other mast cells, as well as to VEGF from tumor cells and immune cells. TAMCs secrete immunosuppressive cytokines such as TGF-β and IL-10, but their more important role in tumor progression is promoting and helping the angiogenic process. TAMCs produce pro-angiogenic factors such as basic fibroblast growth factor (bFGF) and VEGF, ECM modulators such as matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (uPA), as well as chimase, tryptase and histamine. Treatment with compound 48/80, which triggers histamine release, causes an angiogenic response in rats and mice. Despite the importance of TAMCs in tumor progression, their metabolism has not been studied so far. Nevertheless, several studies have been carried out in non-tumoral mast cells.

In 1965, Chakravarty suggested that rat mast cells had higher glycolytic rates than oxidative ones, and some years later he and others pointed out the importance of glucose metabolism and lactate production for histamine release. However, respiration inhibitors block histamine release, and hence energy is necessary for activation and secretion of histamine. On the other hand, two different studies demonstrated the inverse correlation between glutamine metabolism and mast cell function, and tryptophan conversion to kynurenine triggers mast cell degranulation. Kynurenine, in turn, promotes tumor invasion, further demonstrating the association between mast cell function and tumor progression.

More recent works tried to shed some light on the importance of glucose metabolism for mast cell function. Sekar and co-workers studied NO metabolism in mast cells. They demonstrated that NO induced tyrosine nitration of aldolase A, inhibiting this glycolytic enzyme, with the consequent accumulation of fructose 1,6-bisphosphate (FBP). This accumulation inhibited the degranulation of mast cells. Enolase, the ninth enzyme of the glycolytic pathway, has been related with mast cell differentiation, and Chakravarty saw in his studies that treatment with fluoride, an enolase inhibitor, diminished the glucose-supported histamine release. Moreover, inhibition of pyruvate dehydrogenase (PDH), the clue enzyme for the TCA, inhibits mast cell degranulation and cytokine secretion. These last pieces of evidence indicate a glucosedepending mast cell function. However, other works contradict these results. FceRI is a receptor which leads to mast cell degranulation after its ligation with IgE. FceRI has been shown to inhibit PKM2, a process necessary for mast cell degranulation. Accumulation of
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FBP due to the inhibition of this enzyme ceases degranulation of mast cells. Nevertheless, these last authors mention that accumulation of FBP leads to re-activation of PKM2 and reestablishment of glycolytic normal levels, thus inhibiting mast cell function. Furthermore, polyamines have been detected in mast cell granules, and treatment with DFMO diminishes histamine intracellular storage and increases PKM2 expression. This fact establishes a positive relation between polyamine metabolism and degranulation of mast cells with some implication of the glycolytic pathway. Further studies should be performed in order to clarify the exact role of glucose metabolism in mast cell function and its connection with tumor progression.

3.5.2. Tumor-associated pericytes

Pericytes are responsible for morphological and functional abnormalities of tumor blood vessels, and interaction between tumor cells and pericytes has been shown to improve malignancy of glioblastoma. Tumor-associated pericytes present greater migration and proliferation rates than normal ones, and hence they are loosely attached to ECs.

Several studies have been carried out in retinal pericytes in the context of diabetic retinopathy, but without exploring glucose metabolism in pericytes. The only work about pericyte metabolism performed to our knowledge demonstrated that lung pericytes from pulmonary arterial hypertension patients presented higher expression of PDK-1, an inhibitor of PDH, than healthy pericytes. Therefore, it could be considered that normal pericytes display higher rates of OXPHOS than those of glycolysis. Nevertheless, metabolism of tumor-associated pericytes and its relation with tumor progression are yet to be studied.

4. IMPLICATIONS OF TUMOR AND ACCOMPANYING CELLS METABOLISM FOR TUMOR GROWTH AND PROGRESSION

In the previous sections, we have reviewed the main metabolic features of different cells within the TME. However, the complex interplays among these different cells and their metabolic features should be also taken into account. It is well-known that tumor stroma contributes to tumor progression. Several aspects of tumor progression, such as immunosuppression and angiogenesis, depend on the metabolic and signaling pathways involved in them, also orchestrated by interactions of tumor, stromal and immune cells.

4.1. Tumor metabolism and its contribution to immunosuppression
Burnet and Thomas formulated the theory of cancer immunosurveillance (also called immunoediting), according to which lymphocytes would recognize and eliminate tumor cells, thus preventing tumor progression. Nevertheless, some cancer cells are able to escape the immune response by enhancing immunosuppressive activity of immune cells. In fact, escaping immune response has been identified as one of the hallmarks of cancer. Now we know that this immunosuppression is partially controlled by tumor metabolism, and also that of other cells of the TME.

High glucose uptake and lactate secretion have a major role in immunoediting inhibition. As seen above, T cells enhance glycolysis and this improves their effector function. Many types of tumor cells also present a high glycolytic activity, and thereby they avidly consume glucose. As a consequence, low levels of this molecule would be available in the extracellular media for T cells consumption (Fig. 2), and then effector function would be suppressed. An illustrative example is that high HK2 expression in tumor cells mitigates the transcription of the gene coding for IFN-γ, thus contributing to immune response evasion. IFN-γ translation is also regulated by glycolysis through glyceraldehyde 3-phosphate dehydrogenase (GAPDH). When T cells are glucose-restricted, GAPDH becomes available to bind the 3'UTR of IFN-γ mRNA, which results in the inhibition of translation of this cytokine. A similar mechanism occurs with IL-2 (Fig. 2). Furthermore, lactic acid resulting from tumor glycolysis suppresses CTL proliferation, as well as the transcription of IL-2 and IFN-γ, leading to a diminished cytotoxicity of these cells. Probably, a high extracellular level of lactic acid could block the lactic acid export, thus inhibiting further lactate production from glycolysis by T cells. These observations underscore the relevance of aerobic glycolysis for the effector function of T cells. Additionally, Treg cells proliferate in response to TGF-β from tumors. As a matter of fact, Treg cells are the most abundant lymphocytes in the TME. Since their energy metabolism relies on FAO and OXPHOS, they are not as vulnerable to glucose deprivation as effector T cells. In turn, Treg immunosuppressive activity contributes to overall immunosuppression within the TME.

PD-1 is an immunoinhibitory receptor expressed by chronically stimulated T cells. Ahmadzadeh et al., working with metastatic melanoma lesions, found that PD-1 is expressed by TILs at higher levels than those found in normal T cells. Expression of its ligand, PD-L1, has been reported in several human tumors. As PD-1/PD-L1 interaction inhibits T cell proliferation and cytokine production, it could be proposed that TME contributes to a weakened anti-tumor immune response. Different studies have shown that PD-1 expression causes a reduction of glycolysis and a switch to FAO in T cells by suppression of PI3K/Akt. Moreover, recent results have shown that PD-L1 not only inhibits T cell glycolysis but at the same time is able to enhance this pathway in tumor cells through activation of the Akt/mTOR signaling pathway, depriving glucose availability in the TME and thus increasing even further the glycolysis inhibition in these lymphocytes. Therefore, the interaction of PD-1 with its ligand PD-L1 results in an inhibition of effector T cell function (Fig. 2).
It should be emphasized that an increased tumor glycolysis is not the only way to achieve immunosuppression. We have seen that tumors avidly consume glutamine, thus depleting it from the media and affecting the immune response (Fig. 2). Moreover, many tumor cells show high levels of indoleamine-2,3-dioxygenase (IDO1) and tryptophan-2,3-dioxygenase (TDO2), two enzymes that degrade tryptophan to kynurenine. As a consequence, this amino acid is depleted from the media and effector T cells undergo apoptosis (Fig. 2). Kynurenine, as mentioned in another section above, promotes invasiveness by tumor cells (Fig. 3). On the other hand, expression of CD73 in some tumor cells leads to an adenosine accumulation in the extracellular media, which impairs T cell function (Fig. 2). Additionally, NO production by tumor cells leads to anti-tumor immunity, whereas its production by myeloid cells promotes this anti-tumor activity. Since NOS activity requires arginine as a substrate, we dare to ask whether depletion of arginine by tumor cells for the production of NO and polyamines could be the cause to the anti-tumor immunity (Fig. 2). However, combination of L-N\textsuperscript{G}-nitroarginine methyl ester (L-NAME), a NOS inhibitor, with L-argininase has been shown to reduce viability of cancer cells.

In summary, not just tumor aerobic glycolysis, but also amino acid and nucleotide metabolism in tumor cells contribute to the inhibition of a proper T cell function.

4.2. Tumor and endothelial cell metabolism and its role on angiogenesis

As soon as 1971, Judah Folkman proposed that inhibiting angiogenesis could be a new and revolutionary therapy against tumor growth based on his own experimental observations from the sixties. Almost 40 years later, he reviewed the available scientific information regarding a series of different angiogenesis-modulator drugs being developed for the treatment of cancer and other angiogenesis-dependent diseases, therefore reinforcing his early visionary hypothesis and now proposing that angiogenesis could be an organizing principle for drug discovery. There are many factors that are related to angiogenesis (e.g. VEGF, bFGF, HIF-1α, and many others). Many published reviews have already revised this issue along the years. Nevertheless, limitations of anti-angiogenic therapies, mainly based on the inhibition of EC activation by angiogenic factors, especially VEGF, suggested that alternative anti-angiogenic strategies might be considered. The fact that metabolic reprogramming can control angiogenesis opens new horizons to treat this process under pathological conditions through a metabolic approximation and not just by targeting pro-angiogenic molecules. In this section we will focus on the main metabolic features that regulate the angiogenic process, but it should be kept in mind that many other factors may interplay in this scenario.

We mentioned before that glycolysis-derived lactate plays a role in angiogenesis. Végran et al. showed that nuclear factor-κB (NF-κB) is involved in this regulation through PHD inhibition. IL-8 is a pro-angiogenic cytokine expressed by ECs. They
observed that lactate could induce IL-8 expression by these cells in a NF-κB-dependent manner. A sequence of events leading to this is proposed: lactate would be converted to pyruvate by LDH-B, which indirectly inhibits PHD2 by competition with α-ketoglutarate, with the consequent accumulation of IκB kinase (IKK), which phosphorylates inhibitor of kappa B (IκBα), thus liberating the active form of NF-κB and allowing IL-8 transcription. Additionally, PHD inhibition enables the stabilization of HIF-1α and regulation of its target genes expression. These target genes include those coding for pro-angiogenic effectors such as VEGF and for many metabolic enzymes. HIF-1α can also indirectly induce VEGFR2 and bFGF expression. Furthermore, all this requires additionally that ECs incorporate extracellular lactate, secreted by tumor cells, through MCT1 transporters. It has been shown that lactate increases the phosphorylation of Akt, thus promoting the angiogenic process. VEGF plays an additional role, since it promotes fatty acid uptake by ECs, hence contributing to ECs proliferation and angiogenesis. Therefore, lactate uptake by ECs would induce angiogenesis through increased IL-8, VEGF, VEGFR2 and bFGF expression and Akt phosphorylation levels (Fig. 4). Furthermore, it has been seen that extracellular lactate produced by ECs acts as a vasoactive signal for pericytes. It could be possible that lactate secreted by tumor cells could also affect pericyte-mediated vasoconstriction and, thus, angiogenesis.

Moreover, recent studies have uncovered the role of nerve-endothelium interaction on angiogenesis. ECs express β2-adrenergic receptor (ADRβ2), and its deletion leads to inhibition of angiogenesis. More specifically, ADRβ2 blockade in these cells induce a “reverse metabolic switch” towards OXPHOS, by regulation of COX10, a gene related with a cytochrome IV oxidase (Fig. 4).

Finally, it has been recently seen that glutamine and asparagine are essential for angiogenesis. Indeed, glutamine deprivation impairs this process, an effect rescued by the addition of asparagine and α-ketoglutarate. Consequently, inhibiting GLS1 and ASNS activities at the same time seems to be a good anti-angiogenic strategy. Nevertheless, the precise mechanism of these amino acids on the angiogenic switch should be further studied.

### 4.3. Cancer-associated fibroblasts: important assistants for tumor invasiveness

As mentioned above, CAFs rely on enhanced glycolysis. This seems to be due to an enhanced production of ROS by cancer cells. Oxidative stress spreads from cancer cells to adjacent fibroblasts, which reduce their mitochondrial activity and increase glucose uptake, becoming more dependent on aerobic glycolysis (Figs 3 and 4). In a clear example of cell cooperation within TME, CAFs secrete lactate to the media, and this lactate fuels tumor cells, which deliver it to OXPHOS, obtaining energy to sustain their high proliferative rates, in a phenomenon known as “reverse Warburg effect” (Fig. 3).
Likely, this enhanced oxidative stress could induce MCT4 expression in CAFs. Moreover, co-culture of CAFs with MCF7 cells, which mostly rely on oxidative metabolism, results in an increase of MCT1 expression by these tumor cells. Thereby, lactate from CAFs would be incorporated by MCF7 cells, via a lactate shuttle between the stroma (MCT4 in CAFs) and tumor cells (MCT1 in MCF7), in a kind of tumor-feeding mechanism.\textsuperscript{116} Something similar has also been observed in osteosarcoma.\textsuperscript{207}

Lactate secreted by CAFs could have the same effects as those of lactate produced by tumor cells. Romero-Garcia et al. reviewed lactate contribution to the TME. From their review, the following should be highlighted: i) lactate ability to induce MMP-9, an enzyme involved in migration and invasion of cells during the angiogenic process (Fig. 4); ii) immunosuppression; iii) expression of pro-angiogenic factors; and iv) activation of ECs through MCT1.\textsuperscript{208} Several of these processes are regulated, at least in part, by MSF expression in CAFs, a cytokine related to tumor growth.\textsuperscript{115} However, some authors have suggested that the effects caused by extracellular acidification are specific of tumor cells.\textsuperscript{18,120} It has been reported that lactate from cancer cells induce hyaluronic acid production by fibroblasts, contributing to tumor invasiveness (Fig. 3).\textsuperscript{209} In addition to this, CAFs express TGF-β and SDF-1, which confer them their tumor phenotype, due to the activation of the transcriptional regulator heat shock factor 1 (HSF1), as well as pro-angiogenic features.\textsuperscript{115,210,211} Moreover, since Treg cells proliferate in response to TGF-β from tumors, CAF-secreted TGF-β could also help the development of immunosuppression (Fig. 2).\textsuperscript{183} It is well known that CAFs promote tumor progression and invasion, in part by secreting multiple molecules involved in ECM remodeling (Fig. 4).\textsuperscript{212,213} Regarding angiogenesis, several available data suggest a connection between CAFs and tube formation.\textsuperscript{214,215}

Nonetheless, lactate is not the only metabolite from CAFs that fuels tumor cells. Recent studies have shown that CAFs are able to synthetize glutamine from glutamate, aspartate and alanine, and these cells secrete this glutamine, which is used by cancer cells (Fig. 3). Again, tumor cells are not passive, but they secrete glutamate from glutaminolysis as well as the already mentioned lactate, both contributing to glutamine secretion by CAFs.\textsuperscript{120} This interesting GS/GLS intercellular cycle within the TME deserves to be further explored. On the other hand, fatty acids are also synthetized and secreted by CAFs and taken up by breast tumor cells (Fig. 3), favoring tumor progression.\textsuperscript{216} Furthermore, NOS-expressing CAFs support growth of breast and prostate cancer cells, suggesting the relevance of NO metabolism in these cells for tumor progression.\textsuperscript{217}

In summary, CAFs contribute to tumor progression by fueling cancer cells, remodeling the ECM, increasing Treg proliferation and promoting angiogenesis, all in all allowing invasiveness.

\textbf{4.4. Tumor-associated macrophages and tumor progression}
We have seen that TAMs seem to rely on aerobic glycolysis, secreting large amounts of lactate. As a matter of fact, treatment of these cells with 2-deoxyglucose (2-DG) inhibits their pro-metastatic phenotype.\textsuperscript{218} Interestingly, lactate from tumor cells could help by inducing aerobic glycolysis in TAMs through the Akt/mTOR pathway (Fig. 3).\textsuperscript{135} In addition, tumor cell-derived lactate is able to induce TAM polarization by inducing Fizz1, Mgl1 and Mgl2 markers via HIF-1α. Additionally, VEGF and arginase 1 (Arg1) are upregulated in these cells also via HIF-1α.\textsuperscript{219} In the first case, TAMs can be linked to angiogenesis induction. Indeed, a relationship between TAM number and tumor angiogenesis has been documented in breast cancer.\textsuperscript{220} TAMs also produce other molecules involved in the angiogenic process, such as TNF-α, which induces MMP-9 expression, uPA, IL-1, which, through cyclooxygenase 2 (COX2), upregulates HIF-1α, increasing transcription of VEGF in turn, and CCL18.\textsuperscript{221,222} Therefore, it is likely that TAMs help to induce tumor angiogenesis (Fig. 4). This pro-angiogenic effect of TAMs has been already seen, along with immunosuppressive features.\textsuperscript{223}

Regarding metabolism of arginine, Arg1 has an important role in tumor progression, and participates in polyamine production, necessary for collagen synthesis, cell proliferation and tissue remodeling.\textsuperscript{219} Indeed, some evidence hint that TAMs could contribute to tumor invasion by secreting MMPs.\textsuperscript{224} There is some controversy regarding the presence of iNOS expression in TAMs. iNOS is an enzyme that produces NO from arginine. This enzyme is present in M1 macrophages whereas is absent in M2 macrophages.\textsuperscript{225} Regarding metabolic features of these cells, iNOS is able to block OXPHOS while upregulating the glycolytic rate, and therefore iNOS expression corresponds with M1 and TAM metabolic profiles.\textsuperscript{226} Some authors have found iNOS expression in TAMs while others could not.\textsuperscript{227,228} On the other hand, TAMs could have a role in immunosuppression, since depleting extracellular arginine by Arg1 activity would deprive T cells of this amino acid, affecting their proliferation.\textsuperscript{229} Moreover, TAMs express high levels of IDO, producing kynurenine (Fig. 3), and this tryptophan degradation impairs T cell function.\textsuperscript{230} These data reflect the immunosuppressive capacity of TAMs (Fig. 2), and iNOS has an immunosuppressive (as well as anti-angiogenic) effect.\textsuperscript{14} Therefore, additional experiments should be performed in order to confirm the involvement of iNOS in these cells.

Furthermore, these macrophages are unable to produce IL-12, a cytokine required to activate the anti-tumor responses mediated by NK cells, Th1 cells and CTLs. Instead, they produce IL-10, inducing Th2 polarization, and these Th2 cells secrete IL-4, promoting M2 polarization to TAMs in a positive-feedback cycle.\textsuperscript{122} Th2 cells release anti-inflammatory cytokines, so they do not contribute to the anti-tumor immune response. IL-10 secreted by TAMs also increases the number of Treg cells present in epithelial ovarian cancer (Fig. 2).\textsuperscript{231} It has been demonstrated recently that IL-10 inhibits mTOR activation in macrophages, thus leading to a reduction in the glycolytic pathway and ROS liberation from damaged mitochondria.\textsuperscript{232,233} Since mTOR inhibition promotes Treg cell differentiation, a relationship between IL-10 from TAMs and mTOR in tumor progression may be established.\textsuperscript{148}
In addition, as tumor cells, TAMs also express PD-L1, contributing to immunosuppression (Fig. 2). Lactate secreted by cancer cells is able to increase IL-23 secretion by TAMs, a tumor-promoting cytokine involved in the generation of Th17 cells, thus contributing to tumor progression.

Fatty acid and glutamine metabolism in TAMs are also important for tumor progression. For example, an elevated FA biosynthesis, uptake or storage contributes to the pro-tumorigenic profile of these cells. On the other hand, TAMs show high levels of GS expression, thus liberating glutamine to the media for feeding tumor cells and contributing to nitrogen metabolism in these cells, as CAFs do (Fig. 3).

All these facts indicate that TAMs can help tumor cells to evade the immune response, to trigger tumor angiogenesis and to promote invasiveness.

4.5. Other examples of “friendly neighbors” of tumors

Not mentioned above, ECs are able to help tumor cells within the TME. For example, they can extrude mitochondria to tumor cells through tunneling nanotubes and thus they can acquire resistance to chemotherapy (Fig. 3). However, the already mentioned stromal cells are not the only ones able to help tumors to grow. Depending on the type of cancer, there can be other cells that feed tumor cells. They could be called “friendly neighbors”, as in the title of a comment regarding a letter which described the alanine release from pancreatic stellate cells to tumor cells in the pancreas. Some mesenchymal stromal cells have been shown to take up cystine and convert it into cysteine, which is released and taken up by tumor cells from chronic lymphocytic leukemia (CLL). These cancer cells use this cysteine for glutathione (GSH) synthesis, involved in cell survival and resistance to drug cytotoxicity. As CAFs and TAMs do, adipocytes in pancreatic cancer synthetize and secrete glutamine to the media and thus they can feed tumor cells. But that is not all: it has been seen in several types of cancer that adipocytes release fatty acids that are used as fuels by tumor cells, thus contributing to invasiveness, as in the case of CAFs. Moreover, NO-mediated S-nitrosylation triggers adipocyte formation, thus providing tumor cells a source of fatty acids. In addition, adipocytes also secrete arginine that are used by tumor cells to NO synthesis, and the resulting citrulline is taken up by adipocytes in a cross-talk between both cells (Fig. 3).

5. HOST METABOLISM ALTERATIONS AFTER TUMOR DEVELOPMENT

We have already revised some features and implications of the metabolism of the cells within the TME. However, it should not be forgotten that this TME is just a small
part of the organism bearing the tumor. Tumor angiogenesis developed by ECs in the TME allows the secretion of several soluble factors to the circulation, which leads to pathological endocrine effects and an interaction of this microenvironment with the rest of the tissues. So, we cannot just talk about a TME, but a tumor macroenvironment should be as well (or even more importantly) considered, since cancer-associated systemic syndromes develop in this disease.\textsuperscript{246}

The concept of the “systemic effect” was firstly proposed by Shapot. He affirmed that all malignant tumors alter host homeostasis and metabolism even in the absence of metastasis, whereas benign tumors do not share this property.\textsuperscript{247} He distinguished between two manifestations of this systemic effect: i) the alteration of the host metabolism by competence of the tumor with host tissues, and ii) a dysregulation of endocrine gland activities and, therefore, a diminished sensitivity to hormones.\textsuperscript{247} Recently, the concept of solid tumors as systemic metabolic dictators has been proposed.\textsuperscript{248}

The most classical feature of tumors in the context of their interaction with the host is the concept of tumors acting as “nitrogen traps”. As early as 1889, Müller observed a negative nitrogen balance in patients with malignant tumors.\textsuperscript{249} Nevertheless, the concept of nitrogen trap was firstly demonstrated by Mider.\textsuperscript{250} Moreover, because glutamine is the most abundant amino acid in blood, some authors consider tumors as “glutamine traps”.\textsuperscript{251} Early results obtained by our group in Ehrlich ascites tumors suggested that tumors elicit a specific response from the host tissues, so that the whole organism contributes to supply glutamine to the tumor.\textsuperscript{33} Indeed, glutamine content in the host decreases in fast growing tumors due to a flux of glutamine from the host to the tumor, low or null GS activity in the tumor and faster transport of glutamine through the plasma membrane of tumor cells in comparison with non-tumor cells (Fig. 5).\textsuperscript{12,252} There is the exception of some tumors that present a GS upregulation as an adaptation to glutamine depletion, a feature that is not specific to tumor cells.\textsuperscript{253,254} In spite of the importance of glutamine for tumors, changes in concentrations of other amino acids are also observed in plasma after tumor transplantation due to the host-tumor interaction.\textsuperscript{255}

This nitrogen trap may have other effects in the organism. For example, it has been seen that tumors intercept uridine from lymphoid organs, thus inhibiting RNA synthesis, and DNA synthesis is suppressed in the spleen of tumor-bearing mice.\textsuperscript{256,257} Due to the avid host glutamine consumption by the tumor, concentration of glutathione in natural killer cells diminishes, with the consequent loss of activity of these cells.\textsuperscript{258} All these data support that tumors acting as glutamine traps also compromise the immune system response and, therefore, there is an immunosuppression helped by the alteration of nitrogen metabolism in the host (Fig. 5). Some authors have observed that an oral supplement of glutamine in the diet can have benefits in tumor-bearing animals and cancer patients, although a consensus about this has not been achieved.\textsuperscript{259,260}

But tumors not only take nitrogen from the diet. They are also able to take it from host tissues with the consequent body weight loss.\textsuperscript{250,261} However, tumor grows to a
lesser extent when there is no nitrogen available from the diet.\textsuperscript{262} This loss in body weight leads to cancer-associated cachexia.\textsuperscript{263} Nitrogen from host tissues proceeds from protein catabolism, stimulated by an upregulated production of adrenocortical hormones (ACH) resulted from a dysregulation of the endocrine system (Fig. 5).\textsuperscript{264} This dysregulation can lead to other harmful effects in the organism, such as thrombosis and immunosuppression.\textsuperscript{265,266} Now we know that this upregulation of glucocorticoid production is caused by IL-6 secretion from the tumor through inhibition of some hepatic functions such as ketogenesis (Fig. 5).\textsuperscript{267} As a matter of fact, inhibition of IL-6 diminishes tumor growth and cachexia.\textsuperscript{268}

It has been seen that IL-6 from lung adenocarcinoma is able to inhibit another characteristic of liver metabolome, such as hepatic insulin signaling.\textsuperscript{269} This insulin resistance contributes to protein catabolism and induction of glucogenolysis and gluconeogenesis (Fig. 5). Indeed, gluconeogenesis is induced by glucocorticoids after tumor transplantation, and lower levels of glycogen are found in the liver of tumor-bearing animals.\textsuperscript{249,270} Glucose can be synthetized from gluconeogenic amino acids. These amino acids include glutamine, which is used mainly in kidneys, and alanine, used almost exclusively by the liver.\textsuperscript{271} A significant part of this gluconeogenic glutamine comes from catabolism of muscle proteins, which reflects the correlation between cachexia and gluconeogenesis.\textsuperscript{272} Very recently, a study of plasma metabolome from breast cancer patients revealed a positive correlation between lactate, pyruvate and alanine levels, and a negative correlation of pyruvate and alanine with glucose.\textsuperscript{273} This corresponds with the Cori cycle, an inter-system cycle active in tumor patients: lactate released from cancer cells, but also from muscles, goes to the liver, as well as alanine from muscle, and these metabolites are used in gluconeogenesis in that organ, increasing the glucose available for cancer cells and their stroma, and thus enhancing tumor malignancy and associated body weight loss (Fig. 5).\textsuperscript{274} Moreover, the use of amino acids for gluconeogenesis limits the protein synthesis in the host, contributing to vital organs dystrophy.\textsuperscript{249} Indeed, a low amount of membrane-bound ribosomes and a defect of the small subunit of ribosomes in muscle were found in tumor-bearing animals.\textsuperscript{275,276}

Due to the Warburg effect, many tumors depend on aerobic glycolysis. For that reason, tumors can also be considered as "glucose traps".\textsuperscript{249} The consequent decrease in glucose levels due to its consumption by the tumor is, in part, responsible for the up-regulated glycogenolysis and gluconeogenesis. But that is not all. Administration of additional glucose inhibits fatty acid mobilization in the host, showing a modulation of fatty acid metabolism due to glucose depletion caused by the tumor.\textsuperscript{277} As a matter of fact, lipid catabolism in adipocytes promotes cancer-associated cachexia in tumor-bearing mice.\textsuperscript{278} This mobilization of fatty acids could also be associated with fatty acid synthesis in tumors, as serum levels of fatty acids were found to be lower in tumor-bearing mice as compared to the controls (Fig. 5).\textsuperscript{279}
Interestingly, supplementation of arginine in the diet inhibits body weight loss and diminishes tumor growth as well as nitrogen trapped by the tumor. On the one hand, increased leucine oxidation due to additional, available arginine leads to a decrease in protein catabolism. On the other hand, arginine is able to activate the immune system, with the consequent reduction of tumor growth. Nowadays we know the importance of arginine in T cells activity. We would like to highlight the use of arginine for polyamine synthesis, a process enhanced in tumors that could be hence responsible for immunosuppression by depleting extracellular arginine (Fig. 5).

Other amino acids can be taken up by tumors from host tissues. A flux of several essential amino acids, such as valine, leucine, isoleucine, phenylalanine, lysine and arginine, as well as the sulfur amino acid methionine, was observed in Ehrlich carcinoma-bearing mice. Regarding methionine flux, this could be explained by the active polyamine biosynthesis in the tumor, also demonstrated by the observation of a net flux of ornithine from host to tumor and an increase in ODC activity in the seventh day after tumor transplantation in the same animal model (Fig. 5). Moreover, tumors can take cysteine and incorporate it through CD44 in order to synthetize glutathione. It has been seen that CD44 interacts with PKM2, increasing the Warburg effect. Therefore, inhibition of this cell marker leads to an increased glucose oxidation and reduced glutathione levels in tumor cells, enhancing the oxidative damage in these cells.

In addition of inducing protein catabolism in the host and hence acquiring amino acids, Ras-mutant tumor cells are able to incorporate extracellular proteins (mostly serum albumin) by macropinocytosis, and to obtain amino acids from their lysosomal degradation for sustaining cell proliferation even in the absent of extracellular glutamine. Indeed, Holm et al observed that the amount of nitrogen excreted in colorectal cancer was 10-fold higher than the equivalent amino acid uptake, pointing out the possible incorporation of extracellular proteins. PIKfyve has been demonstrated to promote recovery and redistribution of nutrients from vacuoles after lysosomal degradation of engulfed proteins, thus supporting Ras-mutant cell proliferation. On the other hand, an input of amino acids results in mTORC1 activation, which inhibits lysosomal catabolism of extracellular proteins. Besides, oncogene Ras does not only induce macropinocytosis of extracellular proteins, but it also induces lipid scavenging, thus conferring resistance to inhibition of stearoyl-CoA desaturase 1 (SCD1), a key enzyme in fatty acid metabolism. Novel therapeutic strategies are emerging based on these discoveries. For example, drug conjugation with albumin (e.g. paclitaxel) increases intratumoral drug concentration and enhances anti-tumoral activity. mTORC1 inhibitors have sometimes failed in suppressing tumor growth. Combination of mTORC1 inhibitors with blockade of extracellular proteins macropinocytosis or PIKfyve inhibitors could be a promising combined strategy for Ras-mutant tumors.
In summary, host-tumor interactions and the presence of extracellular substrates are of great importance for tumor progression, and metabolism plays an essential role. Despite the relevance of host metabolism in tumors, just a few studies have been performed in the last years, and the vast majority of research regarding this issue is previous to the present century. Therefore, more research would be necessary in order to improve treatment for cancer patients taking into account the whole organism homeostasis.

6. TARGETING METABOLISM OF TUMOR MICROENVIRONMENT CELLS FOR CANCER THERAPY

The “re-discovery” of the Warburg effect and increased glutaminolysis and the identification of tumor metabolism reprogramming as a hallmark of cancer renewed the interest in cancer metabolism after decades of oversight and has led to a renewed interest in targeting tumor metabolism in the last two decades. Many compounds targeting cancer metabolism have been tested in vitro, in vivo and in clinical trials. These compounds include glycolysis inhibitors like 2-DG, lonidamine, 3-bromopyruvate and dichloroacetate and inhibitors of GLS such as 968, BPTES and other glutamine analogues, including DON, acivicin and azaserine, among many others.7,11-13 However, the search for anti-glutamine cancer therapies, despite good results in in vivo models, was soon forgotten.292 A renewed interest in these agents has been recently triggered by the observation that GLS inhibitors may help to overcome acquired resistance to anti-tumor drugs in ovarian and non-small-cell lung cancer.293-296 Inhibiting polyamine metabolism has also been shown to decrease tumor growth, and its targeting is considered of great relevance for cancer therapy.85,297 Additionally, treatment using asparaginase has been proved to be useful against leukemia. Moreover, this enzyme has a well-known immunosuppressor role, that can be explained by an almost undetectable ASNS activity in lymphoid tissues and the glutaminase activity presented in most asparaginases.298-300 Therefore, since treatment with asparagine inhibits T cell activation as well as cytokine production and proper function of M1 macrophages, it should be taken into account that targeting asparagine metabolism in tumors could also affect the immune system.301,302

Furthermore, the concept of “oncometabolites” has opened a new window for tumor treatment. We could define the term oncometabolite as a molecule from normal metabolism that is able to allow tumor progression through its accumulation due to a metabolic dysregulation. The best and first known oncometabolite is 2-HG, which causes changes in gene function in tumors by epigenetic regulation.43 One of the consequences of the accumulation of 2-HG to limit the production of chemokines CXCL9 and CXCL10, so preventing CD8+ T cell recruitment to the tumor, for example.303 In the last years, efforts to inhibit the newly gained function of the mutant IDH enzymes (IDH1 and IDH2) have led to the development of IDH inhibitors which
are already in clinical trials.\textsuperscript{304–306} Other molecules are also considered as oncometabolites, and their targeting should also be researched.\textsuperscript{307,308}

However, in the last years alternatives have emerged with the new understanding of the complex metabolic interactions within the TME. As we have shown above, overall TME metabolic features are sometimes determined by cytokines or pro-angiogenic factors production. In fact, chemoresistance is sometimes enhanced due to interactions with stromal cells and components of the ECM.\textsuperscript{309} On the other hand, it is known that non-tumor cells are genetically more stable than tumor cells, and thus it is less likely that these cells could develop adaptive mutations to treatments.\textsuperscript{224} Therefore, targeting metabolism of TME stromal cells, instead of tumor cell metabolism or in addition to it, could be a promising strategy against tumor progression.

Since metabolism and angiogenesis are related, it could be expected that metabolic modulators were also able to affect different steps of the angiogenic process. Among other examples, 3-bromopyruvate, an inhibitor of hexoquinase, and \(\alpha\)-cyano-4-hydroxyccinnamic acid (CHC), which blocks MCT lactate transporter, inhibit angiogenesis in HUVEC.\textsuperscript{310} 2-DG, the most well-known glycolytic inhibitor, inhibits angiogenesis \textit{in vitro} and \textit{in vivo}.\textsuperscript{311} The glycolytic pathway is not the only possible target. For instance, acivicin, a glutamine analogue, disrupts angiogenesis \textit{in vivo}, and chloroquine, a GDH inhibitor, enhances the anti-angiogenic effect of sunitinib.\textsuperscript{312,313} In addition, some statins, HMG-CoA reductase inhibitors that affect metabolism of cholesterol, and DFMO, an inhibitor of ODC, involved in polyamine metabolism, are capable of suppressing the angiogenic process.\textsuperscript{314–316}

Recently, three articles simultaneously published in \textit{Cell Reports} have demonstrated that the induction of metabolic symbiosis could be responsible for acquired resistance to anti-angiogenic drugs.\textsuperscript{317–319} Treatment with inhibitors of angiogenesis, including sunitinib, may give rise to an extensive vascular collapse that will produce hypoxic and normoxic regions in the tumor. In the hypoxic cancer cells, HIF-1\(\alpha\) induction will upregulate GLUT1 and MCT4, leading to high levels of lactate secretion. This lactate will be imported by the normoxic cancer cells, which express the lactate transporter MCT1, and catabolized with consequent induction of mTOR signaling to promote tumor metabolism. In this way, normoxic cancer cells save glucose for the hypoxic cells and use the lactate produced by hypoxic cells in conjunction with glutamine.\textsuperscript{317} Targeting metabolic symbiosis may therefore be a new strategy to overcome the resistance development to anti-angiogenic therapy in patients.

Targeting EC metabolism could be, as well, a way to inhibit tumor angiogenesis.\textsuperscript{197} Inhibition of PFKFB3 and pharmacological blockade of MCT1 disrupt angiogenesis \textit{in vitro} and \textit{in vivo}, and LDH-A inhibition impairs proliferation of pulmonary microvascular ECs.\textsuperscript{93,199,320} Indeed, taking EC metabolism as a target for modulating pathological angiogenesis may improve chemotherapy, as seen for a PFKFB3 inhibitor, 3-PO, which impairs metastasis without affecting proliferation of tumor cells.\textsuperscript{321} After uncovering the importance of fatty acid metabolism in ECs, targeting fatty acid
synthesis and oxidation is emerging as a novel therapeutic approach to inhibit EC metabolism and angiogenesis.\textsuperscript{106,322} Furthermore, etomoxir, a CPT1a inhibitor, represses angiogenesis.\textsuperscript{106} Glutamine and asparagine metabolism are also emerging targets for inhibition of the angiogenesis process.\textsuperscript{99,100}

Many anti-angiogenic compounds are available and already approved for their use in patients.\textsuperscript{192,323} Moreover, a combinatory strategy is also being explored, since sometimes anti-angiogenic therapy may be not enough to treat tumors.\textsuperscript{5} This anti-angiogenic therapy could result in i) the recovery of the normal perfusion in tissue, with the consequent reduction in hypoxia and an improvement of the immunosupportive immune system, ii) no change or iii) excessive pruning of the vasculature, with a decrease in blood flow and an increase in hypoxia.\textsuperscript{324} Therefore, its combination with metabolic modulators or with immunotherapy could improve the treatment.\textsuperscript{324–326}

The use of inhibitors of lactate transport and production could be a good strategy to target the reverse Warburg effect in stromal cells, and not just lactate metabolism in tumor cells. An inhibitor of MCT1 (AZD3965) is already in phase I trials to this aim.\textsuperscript{327} Similarly, metformin can also be used to target stromal cells in addition of tumor cells. It has been shown that this drug can block lipid accumulation in ovarian cancer cells adjacent to adipocytes, and reverse the malignant phenotype of CAFs by restoring caveolin-1 expression in these cells.\textsuperscript{328,329} Other possibilities are targeting GS in CAFs, as well as GLS in tumor cells, in order to avoid glutamine transfer from CAFs to cancer cells.\textsuperscript{120} Other suggested therapies based on targeting stromal cell metabolism (such as CAFs and CAAs) are collected in the bibliography.\textsuperscript{330}

The denominated checkpoint blockade therapy using antibodies against PD-L1 has emerged as a strategy to restore glucose in the TME and recover T cell effector function in order to suppress tumor progression.\textsuperscript{25} Since tumor and T cells share many metabolic features, targeting their metabolism can have undesired effects. For example, administration of mTOR inhibitors can either promote effector T cells or inhibit them. Furthermore, blocking glycolysis could affect T cell metabolism and lead to a poor prognosis of cancer. However, the use of glycolytic inhibitors before the induction of an immune response may allow T cells to enter a TME with higher glucose concentration, favoring a proper anti-tumor immune response.\textsuperscript{15} Combining an anti-metabolic strategy with a checkpoint blockade therapy could improve the T cell function and cancer prognosis. For example, it has been reported that targeting CD73 in tumors enhances the efficacy of anti-PD-1 and anti-CTLA-4 treatments.\textsuperscript{331}

Anti-tumor T cell function can be also partially recovered by inhibiting Arg1 with tadalafil.\textsuperscript{14} Inhibitors of IDO have been proposed to restore T cell proliferation and cytokine production, and dimethylfumarate (DMF), an anti-angiogenic compound, is able to inhibit IDO activity in human immune cells.\textsuperscript{17,332,333} Moreover, very recently an inhibitor of IDO, erianin, has also been shown to inhibit tumor angiogenesis.\textsuperscript{334}
In summary, targeting stromal cell metabolism and development of immunotherapy with metabolism as a target may improve cancer therapies by inhibiting angiogenesis and recovering anti-tumor immune response, leading to tumor regression. Several compounds able to modulate metabolic features with proved anti-tumor activity are collected in Table 1. However, it is always important to be careful with secondary effects and to make sure that normal metabolism is not affected by the treatment. Further research will be necessary to progress on cancer treatment via inhibition of the TME metabolism.

7. CONCLUDING REMARKS AND OUTSTANDING QUESTIONS

In this review we have tried to explore metabolism within the TME and how it affects tumor growth and progression. Four major kinds of cells have been analyzed: ECs, TILs, CAFs and TAMs, apart from tumor cells. Summarizing, all these cells rely mainly on aerobic glycolysis with the exception of Treg cells, which mainly depend on an oxidative metabolism. Lactate production by tumor cells would contribute to promote tumor angiogenesis via NF-κB and HIF-1α stabilization. TAMs and CAFs also collaborate by secreting pro-angiogenic factors. During tumor progression a process termed immunosuppression occurs, by which T cells are unable to exert a proper anti-tumor immune response. Tumor cells, by glucose competition and lactate secretion, as well as other metabolic features of these and other cells, are responsible for this. PD-1/PD-L1 interaction is also a way to immunosuppression, in which tumor cells, T cells and TAMs are implicated. CAFs also fuel tumor cells by a phenomenon called reverse Warburg effect and by glutamine synthesis and secretion, along with TAMs and CAAs.

Although in this review we have focused on the changes regarding metabolism in the TME, metabolism is considered a complex and dynamic network able to adapt in response to shifts and metabolic demands. Therefore, cancer metabolic reprogramming is just an example of the flexibility and adaptability of metabolism. Circadian rhythms, hypoxia, exercise, hibernation period and many other factors are able to modulate gene expression and metabolic features of healthy cells. The lactate shuttle between tumor cells and other cells of their microenvironment is also present in healthy tissues, such as muscle and brain. Moreover, it has been recently demonstrated that there are also changes in metabolism during developmental progression and not just during differentiation, and a loss of metabolic flexibility could lead to pathologies associated to metabolic syndrome. Actually, this metabolic flexibility is not only found in animals, but in all organisms. Plants, for example, are able to modify their metabolism in response to environmental stress. Due to this metabolic flexibility, tumors can modulate the metabolism of the tissues in the so-called systemic effect. Therefore, not only metabolism of the sole TME, but also the changes in the metabolism of the whole organism triggered by the tumor should be studied.
In conclusion, although it is obvious and well-documented that there is a metabolic switch during tumor progression, these kinds of changes also take place in healthy tissues as a normal process or under particular situations and they should not be considered as surprising. All in all, cancer metabolic reprogramming ought to be studied as an ordinary and expected feature of metabolism. Regarding possible therapies, targeting the metabolic features of the different cells of the TME, or putting the target in the angiogenic process or the immune system, will allow us to design new strategies to fight cancer in combination with classical metabolic approaches.

We could take into consideration the next remarkable aspects: i) aerobic glycolysis is upregulated in different cells of the TME, except for Treg cells; ii) tumor cells should be classified as oxidative and glycolytic ones, even within the same tumor; iii) due to different metabolic modulations, cells of the TME help to tumor progression, affecting invasiveness, angiogenesis and immunosuppression; iv) tumor macroenvironment should not be rotten in oblivion, and more research should be performed in order to improve treatments; v) metabolism regulates and is linked to many other physiological characteristics, being part of an interconnected network; vi) the concept of metabolic switch is not specific of cancer, but an example of the global flexibility of metabolism.

Finally, we bring together some questions that remain up in the air waiting for being elucidated: i) Is there any glucose competition between tumor and ECs? And between tumor, CAFs and TAMs? ii) What is the exact mechanism by which lactate undermines T cells glycolytic metabolism? iii) What is the exact role of arginine in the immune system? iv) Which metabolic features characterize TAMCs and tumor-associated pericytes? What is their role in tumor progression? Further investigation will be needed to solve these inquiries.

NOTES ADDED IN PROOF

During the revision period of this article a study showing an interaction between metabolic reprogramming and transcriptional regulation has been published. Dasgupta et al. have shown that the metabolic enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 (PFKFB4) regulates transcriptional programming by activating the oncogenic steroid receptor coactivator-3 (SRC-3) through its phosphorylation at serine 857. An active glucose metabolism allows this phosphorylation, which leads to upregulation of some of the key enzymes of the pentose phosphate pathway (PPP). This activation of purine metabolism is essential for tumor growth and metastasis in breast cancer models, since ablation of SRC-3 or PFKFB4 leads to a decrease in cell growth and the metastatic progression of the disease. Another enzyme of the same family, PFKFB3, was shown to be involved in angiogenesis. Hence, we would like to remark the importance of metabolism in the development of diseases such as cancer and angiogenic-dependent pathologies through different mechanisms.
We have also become aware of the approval by FDA of enasidenib for the treatment of oncologic patients with tumor \textit{IDH2} gene mutations.\textsuperscript{432}

\textbf{References}


68. Liu W, Le A, Hancock C, et al. Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by


119. Rattigan YI, Patel BB, Ackerstaff E, et al. Lactate is a mediator of metabolic cooperation between stromal carcinoma associated fibroblasts and glycolytic


160. Yoshizaki K, Arizono N, Hayano T, Watari H. Allergen-induced histamine


167. Ryu SY, Hong GU, Kim DY, Ro JY. Enolase 1 and calreticulin regulate the differentiation and function of mouse mast cells. *Cell Signal* 2012;24:60–70.


172. Caspani EM, Crossley PH, Redondo-Garcia C, Martinez S. Glioblastoma: A


184. Ahmadzadeh M, Johnson LA, Heemskerk B, et al. Tumor antigen – specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired Tumor antigen – specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood* 2009;114:1537–1544.

185. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-


578.


263. De Lerma Barbaro A. The complex liaison between cachexia and tumor burden.


288. Palm W, Park Y, Wright K, Pavlova NN, Tuveson, David A, Thompson CB. The utilization of extracellular proteins as nutrients is suppressed by mTORC1. *Cell*


AUTHOR BIOSKETCHES

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Mª Carmen Ocaña, BS in Biology and Master in Cell and Molecular Biology by the University of Málaga (Spain) in 2014 and 2016, respectively, is a PhD student in the Molecular Biology and Biochemistry Department at University of Málaga (Spain). She is currently studying the metabolism of endothelial cells and its relationship with the angiogenic process. Moreover, she is testing the capacity of different compounds to affect metabolism, angiogenesis and/or inflammation. She is also interested in studying metabolism of endothelial cells in the context of the tumor microenvironment.

Beatriz Martínez Poveda

Beatriz Martínez Poveda was graduated in Biology at the University of Malaga (Spain) in 2002 and she achieved her international PhD at the same University in 2007 working on characterization of new natural compounds with anti-angiogenic potential. Then she moved to Madrid for a first post-doctoral period in the Biomedical Research Institute (IIB, Madrid), focusing on the study of hypoxia and anti-angiogenic therapy in tumors using in vivo imaging techniques. In 2009, she started a second post-doctoral period in the Cardiovascular Research National Institute (CNIC, Madrid) working mainly in the molecular characterization of cardiovascular diseases, with significant contributions in the field of aortic valve stenosis and calcification, atherosclerosis and left ventricle non-compaction. Since 2015 Dr. Martínez-Poveda is working as Assistant Professor in the Department of Molecular Biology and Biochemistry at the University of Málaga, and as a post-doctoral researcher in projects related to tumoral angiogenesis and inflammation.

Ana Rodríguez Quesada

Ana R. Quesada graduated in chemistry at the University of Granada (Spain) in 1982 and obtained her Ph.D. in Biochemistry at University of Malaga (Spain) in 1987. She was visiting scientist at the University of Bristol (UK) in 1987 and at the UWM (Wisconsin, USA) in 1991. After working seven years in research departments of pharmaceutical companies, she moved to the University of Malaga in 2004, where she is holding a Full Professor position at the Department of Molecular Biology and Biochemistry. Ana has a special interest in the search and characterization of new anti-angiogenic drug candidates.

Miguel Ángel Medina

Miguel Ángel Medina graduated in Biology at the University of Málaga (Spain) in 1985, where he later obtained his Ph.D. in Biochemistry in 1989. He has been visiting scientist at McGill University (Canada, 1987), the Max Planck Institut für Ernährungspysiology (Dortmund, Germany, 1989), the University of Heidelberg (Germany, 1992) and the Max Planck Institut für biophysikalisches-Chemie (Göttingen, Germany, 2001). He was Assistant (1990-94) and Associate (1995-2009) Professor and
he is currently holding a Full Professor position (2009-today) at the Department of Molecular Biology and Biochemistry (University of Málaga, Spain). Miguel Ángel has a special interest in the search and characterization of new anti-angiogenic drug candidates. Other research interests include cancer metabolism, systems biology, metabolic modeling and rare diseases. He is the (co)-author of around 200 articles in international science journals.
Table 1. Metabolic modulators with proved anti-tumor activity.

<table>
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<th>Target</th>
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</tr>
<tr>
<td>CPT1</td>
<td>Aminocarnitine, Etomoxir, Perhexiline, Ranolazine</td>
<td>Perhexiline and ranolazine are approved for use as an anti-angina therapy.</td>
<td></td>
</tr>
<tr>
<td><strong>Lipid synthesis</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>FAS</td>
<td>C75, Cerulenin, Orlistat, TVB-2640</td>
<td>Orlistat is approved for the treatment of obesity. TVB-2640 is in Phase II of clinical trials (multiple kinds of cancer).</td>
<td></td>
</tr>
<tr>
<td>ACL</td>
<td>Hydroxycitrate, SB-204990</td>
<td></td>
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<tr>
<td>ACC</td>
<td>TOFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline kinase</td>
<td>CK37, MN58b, RSM932A, TCD-717</td>
<td>TCD-717 is in Phase I of clinical trials (advanced solid tumors).</td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>Triacsin C</td>
<td></td>
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<tr>
<td><strong>Mevalonate pathway</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HMGCR</td>
<td>Statins</td>
<td>Approved for the treatment of hypercholesterolaemia</td>
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<tr>
<td><strong>Pentose phosphate pathway</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G6PDH</td>
<td>6-amino nicotinamide, DHEA, DMF, EGCG</td>
<td>EGCG is in clinical trials (multiple kinds of cancer). Dimethyl fumarate is FDA approved (multiple kinds of cancer).</td>
<td></td>
</tr>
<tr>
<td>Metabolism</td>
<td>Enzyme/Drug</td>
<td>Details</td>
<td></td>
</tr>
<tr>
<td>------------------------------------</td>
<td>--------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
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<tr>
<td><strong>Amino acid metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine availability</td>
<td>Pegylated arginine deiminase (ADI-PEG20) rhArg1-PEG (BCT-100)</td>
<td>BCT-100 is in Phase II of clinical trials (multiple kinds of cancer). ADI-PEG20 is in clinical trials (multiple kinds of cancer).</td>
<td></td>
</tr>
<tr>
<td>Arginase</td>
<td>Tadalafil (Cialis)</td>
<td>FDA approved for the treatment of benign prostatic hypertrophy.</td>
<td></td>
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<tr>
<td>IDO</td>
<td>I-methyl-tryptophan (Indoximod) DMF Epacadostat Erianin</td>
<td>Indoximod and epacadostat are in clinical trials (multiple kinds of cancer). Dimethylfumarate is FDA approved (multiple sclerosis).</td>
<td></td>
</tr>
<tr>
<td><strong>Polyamine metabolism</strong></td>
<td>ODC DFMO Phase II of clinical trials (neuroblastoma).</td>
<td></td>
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<tr>
<td>AMD1</td>
<td>MGBG SAM486A MGBG is toxic for clinical development.</td>
<td></td>
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<tr>
<td>Polyamine transport</td>
<td>AMXT-1501</td>
<td></td>
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<tr>
<td>Aminopropyltransferases</td>
<td>AdoDATAD AdoDATO</td>
<td></td>
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<tr>
<td>Polyamine analogs</td>
<td>BENSpm CPENSpm PG-11047 PG-11093</td>
<td>PG-11047 is in Phase I of clinical trials (advanced refractory solid tumors and lymphoma).</td>
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<tr>
<td><strong>Nucleid acid synthesis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme</td>
<td>Inhibitors</td>
<td>Activity</td>
<td></td>
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<tr>
<td>--------------------------------------------</td>
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<td>--------------------------------------------------------------------------</td>
<td></td>
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<tr>
<td>DHFR</td>
<td>Methotrexate</td>
<td>Methotrexate is FDA approved for treatment of cancer, autoimmune diseases, ectopic pregnancy, and for medical abortions. Pemetrexed is FDA approved for the treatment of pleural mesothelioma and non-small cell lung cancer. Pralatrexate is FDA approved relapsed or refractory peripheral T-cell lymphoma.</td>
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<tr>
<td></td>
<td>Pemetrexed</td>
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<tr>
<td></td>
<td>Pralatrexate</td>
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<tr>
<td></td>
<td>Trimitrexate</td>
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<tr>
<td></td>
<td>(antifolates)</td>
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<tr>
<td>Thymidylate synthase</td>
<td>5-fluorouracil</td>
<td>5-fluorouracil is FDA approved for the treatment of several kinds of cancer. Raltitrexed is in Phase IV of clinical trials (multiple kinds of cancer).</td>
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</tr>
<tr>
<td></td>
<td>Raltitrexed</td>
<td></td>
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<tr>
<td>Adenine/adenosine deaminase</td>
<td>Cladribine</td>
<td>FDA approved for the treatment of hairy cell leukemia and B-cell chronic lymphocytic leukemia.</td>
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<td></td>
<td>Fludarabine</td>
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<tr>
<td></td>
<td>Gencitabine</td>
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<tr>
<td></td>
<td>Hydroxyurea</td>
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</tbody>
</table>
Hydroxyurea is FDS approved for the treatment of sickle-cell disease, chronic myelogenous leukemia, cervical cancer, and polycythemia vera.

<table>
<thead>
<tr>
<th>Nitric oxide metabolism</th>
<th>NOS</th>
<th>L-NAME</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Metabolic signaling pathways</th>
<th>HIF-1</th>
<th>Digoxin, Irinotecan, PX478, Topotecan</th>
</tr>
</thead>
</table>
|                            |       | PX478 is in Phase I of clinical trials (advanced solid tumors and lymphoma).
|                            |       | Digoxin is FDA approved for the treatment of several heart diseases.
|                            |       | Irinotecan is FDA approved for the treatment of colon and small cell lung cancer.
|                            |       | Topotecan is FDA approved for the treatment of several kinds of cancer. |

<table>
<thead>
<tr>
<th>mTOR</th>
<th>Everolimus, PP242, Temsirolimus</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Everolimus and temsirolimus are also approved immunosuppressants. Everolimus is approved for the treatment of advanced kidney cancer.</td>
</tr>
</tbody>
</table>

2-DG, 2-deoxyglucose; ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; ACS, acyl-CoA synthetase; AdoDATAD, S-adenosyl-1,12-diamino-3-thio-9-azadodecane; AdoDATO, S-adenosyl-3-thio-1,8-diaminooctane; AMD1, adenosylmethionine decarboxylase; AOA, aminooxyacetate; BPTES, bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide; CHC, α-cyano-4-hydroxycinnamic acid; CPT1, carnitine palmitoyltransferase 1; DCA, dichloroacetate; DFMO, difluoromethylornithine; DHEA,
dehydroepiandrosterone; DHFR, dihydrofolate reductase; DMF, dimethylfumarate; DON, 6-diazo-5-oxo-L-norleucine; EGCG, epigallocatechin gallate; FAS, fatty acid synthase; γ-FBP, γ-glutamate binding protein; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; GLS1, glutaminase; G6PDH, glucose-6-phosphate dehydrogenase; GPNA, L-γ-glutamyl-p-nitroanilide; GLUD, glutamate dehydrogenase; HIF-1, hypoxia-inducible factor 1; HMGCR, HMG-CoA reductase; IDH, isocitrate dehydrogenases; IDO, indoleamine-2,3-dioxygenase; KGDH, α-ketoglutarate dehydrogenase; LDH-A, lactate dehydrogenase A; L-NAME, L-NG-nitroarginine methyl ester; MGBG, methylglyoxal(bis)guanylhydrazone; MPC, mitochondrial pyruvate carrier; mTOR, mammalian target of rapamycin; NHI, N-hydroxy-2-carboxy-substituted indoles; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; PFKFB3, phosphofructokinase-2/fructose-2,6-bisphosphatase 3; PGAM1, phosphoglycerate mutase; PKM2, pyruvate kinase M2; TCA, tricarboxylic acid cycle.

aClinicalTrials.gov Identifier: NCT00487721; bpancreatic cancer, phase II, ClinicalTrials.gov Identifier: NCT00192842; breast cancer, phase II, ClinicalTrials.gov Identifier: NCT01042938; endometrial carcinoma, phase II, ClinicalTrials.gov Identifier: NCT02017353; head and neck cancer, early phase I, ClinicalTrials.gov Identifier: NCT01160302; pancreatic cancer, phase II, ClinicalTrials.gov Identifier: NCT00094445; colorectal cancer, phase I, ClinicalTrials.gov Identifier: NCT00027495; multiple myeloma, ClinicalTrials.gov Identifier: NCT00113841; prostate cancer, phase III, ClinicalTrials.gov Identifier: NCT00096707; lung cancer, breast cancer, pancreatic cancer, gastric cancer and head and neck cancer, phase II, ClinicalTrials.gov Identifier: NCT00848016; leukemia, phase II, ClinicalTrials.gov Identifier: NCT00540722; lymphoma, phase II, ClinicalTrials.gov Identifier: NCT00274531; adrenocortical carcinoma, phase II, ClinicalTrials.gov Identifier: NCT00848016; leukemia, phase II, ClinicalTrials.gov Identifier: NCT00376948; colorectal cancer, phase II, ClinicalTrials.gov Identifier: NCT01985763; ClinicalTrials.gov Identifier: NCT00435448; prostate cancer, phase II, ClinicalTrials.gov Identifier: NCT00633087; lung cancer, breast cancer, pancreatic cancer, gastric cancer and head and neck cancer, phase I, ClinicalTrials.gov Identifier: NCT00967607; adult glioblastoma, phase II, ClinicalTrials.gov Identifier: NCT00540722; lymphoma, phase II, ClinicalTrials.gov Identifier: NCT00275431; adrenocortical carcinoma, phase II, ClinicalTrials.gov Identifier: NCT00848016; leukemia, phase II, ClinicalTrials.gov Identifier: NCT00286780; laryngeal cancer, phase II, ClinicalTrials.gov Identifier: NCT01633541; small cell lung cancer, phase II, ClinicalTrials.gov Identifier: NCT00773955; prostate cancer, phase II, ClinicalTrials.gov Identifier: NCT00666666; ClinicalTrials.gov Identifier: NCT01791595; small cell lung cancer, phase I, ClinicalTrials.gov Identifier: NCT01931787; pancreatic cancer, phase I, ClinicalTrials.gov Identifier: NCT01839981; colorectal cancer, phase I, ClinicalTrials.gov Identifier: NCT02232152; adult acute myeloid leukemia, phase I, ClinicalTrials.gov Identifier: NCT01768897; lymphoma, phase I, ClinicalTrials.gov Identifier: NCT02168140; ClinicalTrials.gov Identifier:
NCT03173248; ClinicalTrials.gov Identifier: NCT02577406; ClinicalTrials.gov Identifier: NCT03026517; colorectal cancer, phase II, ClinicalTrials.gov Identifier: NCT02861300; lymphoma, phase I, ClinicalTrials.gov Identifier: NCT02071888; leukemia, phase I, ClinicalTrials.gov Identifier: NCT02071927; breast cancer, phase II, ClinicalTrials.gov Identifier: NCT03057600; renal cell carcinoma, phase II, ClinicalTrials.gov Identifier: NCT03428217; colon cancer, early phase I, ClinicalTrials.gov Identifier: NCT02891538; bladder cancer, phase II, ClinicalTrials.gov Identifier: NCT00666562; breast cancer, phase II, ClinicalTrials.gov Identifier: NCT00917735; prostate cancer, phase II, ClinicalTrials.gov Identifier: NCT00676780; breast cancer, phase II, ClinicalTrials.gov Identifier: NCT03179904; colon cancer, phase I, ClinicalTrials.gov Identifier: NCT02980029; astrocytoma, phase II, ClinicalTrials.gov Identifier: NCT03032484; melanoma, phase II, ClinicalTrials.gov Identifier: NCT01215864; hepatocellular carcinoma, phase II, ClinicalTrials.gov Identifier: NCT01092091; leukemia, phase II, ClinicalTrials.gov Identifier: NCT02899286; renal cell carcinoma, melanoma and prostate adenocarcinoma, phase I, ClinicalTrials.gov Identifier: NCT02285101; melanoma, phase II, ClinicalTrials.gov Identifier: NCT00520299; prostate cancer, phase I, ClinicalTrials.gov Identifier: NCT01497925; breast cancer, phase I, ClinicalTrials.gov Identifier: NCT01948843; acute myeloid leukemia, phase I, ClinicalTrials.gov Identifier: NCT02875093; hepatocellular carcinoma, phase III, ClinicalTrials.gov Identifier: NCT01287585; glioblastoma, phase II, ClinicalTrials.gov Identifier: NCT02052648; pancreatic cancer, phase II, ClinicalTrials.gov Identifier: NCT02077881; prostate cancer, phase II, ClinicalTrials.gov Identifier: NCT01560923; melanoma, phase III, ClinicalTrials.gov Identifier: NCT03301636; acute myeloid leukemia, phase II, ClinicalTrials.gov Identifier: NCT02835729; sarcoma, phase II, ClinicalTrials.gov Identifier: NCT03414229; lymphoma and solid tumors, phase II, ClinicalTrials.gov Identifier: NCT03322384; renal cell carcinoma, phase III, ClinicalTrials.gov Identifier: NCT03260894; urothelial cancer, phase III, ClinicalTrials.gov Identifier: NCT03374488; head and neck cancer, phase III, ClinicalTrials.gov Identifier: NCT03342352; lung cancer, phase III, ClinicalTrials.gov Identifier: NCT03322566; pancreatic cancer, phase II, ClinicalTrials.gov Identifier: NCT03006302; prostate cancer, phase II, ClinicalTrials.gov Identifier: NCT03493945; ovarian cancer, phase I, ClinicalTrials.gov Identifier: NCT02118285; sarcoma, phase II, ClinicalTrials.gov Identifier: NCT00705653; lymphoma, phase I, ClinicalTrials.gov Identifier: NCT00293488; head and neck cancer, phase IV, ClinicalTrials.gov Identifier: NCT03196843; nasopharyngeal carcinoma, phase II, ClinicalTrials.gov Identifier: NCT02562599; childhood leukemia, phase I, ClinicalTrials.gov Identifier: NCT00003528; gastric cancer, phase II, ClinicalTrials.gov Identifier: NCT03392103; colorectal cancer, phase IV, ClinicalTrials.gov Identifier: NCT01959061; *enasidenib has already been approved by FDA (see Notes added in proof).
FIGURE CAPTIONS

Figure 1. Important aspects regarding metabolism of tumor cells and several cells of the tumor microenvironment.

Figure 2. Role of different cells of the tumor microenvironment in immunosuppression. Different cells of the tumor microenvironment are able to affect the immune activity. Proliferation of Treg cells is modulated by TGF-β from cancer-associated fibroblasts (CAFs) and tumor cells and by IL-10 secreted by tumor-associated macrophages (TAMs). Tumor cells consume high amounts of tryptophan and arginine, thus depleting them from the media. TAMs also consume tryptophan, and HIF-1α induces the expression of arginase 1 (Arg1), hence diminishing arginine concentration in the extracellular media. Part of the arginine consumed by tumor cells can be leaded to nitric oxide (NO) synthesis, which inhibits effector T cells activity. Additionally, the high uptake of glutamine by tumor cells decreases glutamine availability in the media, inhibiting glutaminolysis in effector T cells, which, in turn, impairs polyamine and nucleotide synthesis in these cells. Tumor cells also express CD73 marker, responsible for increasing AMP concentration in the media, which will be converted to adenosine, capable of inhibiting immune response by effector T cells. Regarding glucose metabolism, TAMs and tumor cells express PD-L1, the ligand for PD-1, and their interaction inhibits glycolysis in effector T cells. PD-L1 favors the high glycolytic rate in tumor cells, thus depleting glucose from the media, and then the transcription of IFN-γ and IL-2 is inhibited. All these facts lead to immunosuppression. Solid arrows show production or secretion; dashed arrows represent induction or inhibition; dotdashed arrows indicate a substrate or process integrated to another process; thicker arrows depict a higher rate of incorporation of the indicated substrate.

Figure 3. Metabolite exchange between tumor cells and different cells of the tumor microenvironment and its relation with tumor progression. There are multiple metabolic interactions between the different cells of the tumor microenvironment. For example, endothelial cells (ECs) consume lactate produced by tumor cells, thus enhancing the angiogenic process, and ECs extrude mitochondria to tumor cells, conferring them chemoresistance. Lactate from tumor cells are also consumed by tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs). On the one hand, in TAMs, lactate stabilizes HIF-1α, thus promoting angiogenesis and immunosuppression. On the other hand, in CAFs lactate induces hyaluronic acid production, which contributes to tumor invasiveness along with kynurenine, a tryptophan metabolite produced by tumor cells and TAMs. Lactate production by CAFs is also promoted by ROS liberation from tumor cells. Additionally, cancer-associated adipocytes (CAAs), TAMs and CAFs synthetize glutamine, which is uptaken by tumor cells. CAAs and CAFs also provide fatty acids (FAs) to tumor cells. Moreover, CAAs supply tumor cells with citrulline and arginine, hence contributing to polyamine and nitric oxide (NO) synthesis in these cells. Solid arrows show production or secretion; dashed arrows represent induction or inhibition; dotdashed arrows indicate a substrate or process.
integrated to another process.

**Figure 4.** Role of different cells of the tumor microenvironment in promoting angiogenesis. Tumor cells contribute to activation of angiogenesis through lactate secretion to the media, which is consumed by endothelial cells (ECs). ECs are also able to produce lactate via glycolysis, and this lactate promotes the phosphorylation of Akt, which, in turn, promotes the glycolytic process in a positive feed-back. Indirectly, lactate inhibits prolyl hydroxylases (PHD). PHD inhibition enables stabilization of HIF-1α and the liberation of the active form of NF-κB, thus allowing the transcription of pro-angiogenic factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor receptor 2 (VEGFR2), vascular endothelial growth factor (VEGF) and interleukin 8 (IL-8). VEGF, as well, promotes fatty acid (FA) uptake in ECs. Oxidation of these fatty acids leads to nucleotide synthesis, increasing EC proliferation. Moreover, expression of β2-adrenergic receptor (ADRβ2) favors the glycolytic phenotype through inhibition of OXPHOS. Additionally, other cells of the tumor microenvironment are also able to modulate angiogenesis. For example, stabilization of HIF-1α by ROS liberation from tumors increases the glycolytic rate in cancer-associated fibroblasts (CAFs), and the resulting lactate promotes the liberation of metalloproteinase-9 (MMP9) to the media. Furthermore, TGF-β expressed in these cells activates urokinase-type plasminogen activator (uPA). Both molecules are involved in extracellular matrix degradation. On the other hand, tumor-associated macrofages (TAMs) produce TNF-α, which allows the expression of MMP9 and uPA as well, and of IL-1, which upregulates HIF-1α, hence increasing transcription of VEGF and other pro-angiogenic factors. It has to be taken into account that many other factors produced by the different cells of the microenvironment regulate the angiogenic process, but they are not represented here for the sake of clarity. Solid arrows show production or secretion; dashed arrows represent induction or inhibition; dotdashed arrows indicate a substrate or process integrated to another process.

**Figure 5.** Interactions between tumor and host metabolism. Tumor growth is promoted by means of different metabolic interactions of tumor with host tissues. Tumors secrete IL-6, which has two effects on the liver: i) inhibiting ketogenesis, which stimulates the secretion of adrenocortical hormones (ACH), therefore promoting protein catabolism in muscles, which results in free amino acids for their use by the tumor, and ii) promoting insulin liberation, which induces gluconeogenesis in the liver, thus supplying the tumor with glucose. In addition, gluconeogenesis in the liver also uses alanine from muscles and lactate from muscles and the tumor (all this corresponding to the so-called Cori cycle), and gluconeogenesis is also carried out in the kidneys. Moreover, tumors act as “nitrogen traps”, consuming high amounts of glutamine from the blood. Liver and kidneys have a high glutamine synthetase (GS) and a low glutaminase (GLS) expression, and muscles present high GS expression, thus providing tumors with glutamine. This high uptake of glutamine by the tumor decreases glutamine available for natural-killer (NK) cells, thus diminishing glutathione (GSH) concentration and affecting NK cells activity. Tumors also consume arginine, depleting the arginine
available for other tissues. In addition, tumors take up uridine from lymphoid organs, leading to a decrease in RNA synthesis in these organs. All this contributes to immunosuppression. The arginine consumed can be used for nitric oxide (NO) and polyamine synthesis, helped by a high uptake of ornithine and methionine from the tissues, as well as a high ornithine decarboxylase (ODC) activity. Besides, lipid catabolism is promoted in the adipose tissue, thus liberating free fatty acids (FAs) to the blood that are uptaken by the tumor. Solid arrows show production or secretion; dashed arrows represent induction or inhibition; dotdashed arrows indicate a substrate or process integrated to another process; thicker arrows depict a higher rate of incorporation of the indicated substrate.
<table>
<thead>
<tr>
<th>Tumor cell</th>
<th>Endothelial cell</th>
<th>CAF</th>
<th>TAM</th>
<th>Treg</th>
<th>Effector T cell</th>
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</thead>
<tbody>
<tr>
<td>NO metabolism</td>
<td>↑ Glycolysis</td>
<td>↓ IDH-3α → HIF-1α stabilization</td>
<td>↑ Glycolysis</td>
<td>↑ FAO</td>
<td>↓ Glc → OXPHOS</td>
</tr>
<tr>
<td>↑ Glycolysis</td>
<td>↑ PPP → NADPH &gt; NO and → iNOS</td>
<td>↑ Glycolysis</td>
<td>↑ Glu metabolism</td>
<td>↑ OXPHOS</td>
<td>↓ cytokine production</td>
</tr>
<tr>
<td>↑ PPP → nucleotide synthesis and NADPH source</td>
<td>↑ HBP → N-glycosylation of UTPSFR</td>
<td>MSF → Akt → mTOR</td>
<td>↓ FAO</td>
<td>↑ de novo lipogenesis</td>
<td>↑ PCK-1 → TCR</td>
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<tr>
<td>↑ FAS → lipid synthesis</td>
<td>↑ Glutaminolysis</td>
<td>Lactate oxidation</td>
<td>↑ Eicosanoid production</td>
<td>↑ Glycolysis</td>
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<tr>
<td>↑ Glutaminolysis and ↓ GS</td>
<td>↑ ASNS</td>
<td>↑ GS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ OXPHOS in some tumors or in certain localizations</td>
<td>↑ Polyamine synthesis</td>
<td>Higher metabolic activity than healthy fibroblasts</td>
<td></td>
<td>Nucleotide and polyamine synthesis</td>
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<tr>
<td>Asn, Ser and Gln metabolism</td>
<td>↓ OXPHOS</td>
<td></td>
<td></td>
<td>Arg metabolism</td>
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<tr>
<td>↑ Polyamine synthesis</td>
<td>↑ FAO → dNTPs</td>
<td></td>
<td></td>
<td>proliferation</td>
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152x117mm (300 x 300 DPI)
Immunosuppression

CAF
TGF-β
proliferation

Tumor cell
kynurenine
Polyamine synthesis

Glycolysis
Glc
lactate

CD73
Ade
AMP

Gln

NO synthesis
Arg

PD-1

PD-L1

Trp

Arg1

HIF-1α

IL-10

TAM

Trp

TGF-β

Polyamine synthesis
Nucleotide synthesis

GAPDH

Glutaminolysis

Effector T cell

IFN-γ
IL-2

152x111mm (300 x 300 DPI)
152x112mm (300 x 300 DPI)