Metabolic heterogeneity and immunocompetence of infiltrating immune cells in the breast cancer microenvironment (Review)

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Abstract. Breast cancer is one of the most common malignancies in women and is characterized by active immunogenicity. Immune cell infiltration plays an important role in the development of breast cancer. The degree of infiltration influences both the response to and effect of treatment. However, immune infiltration is a complex process. Differences in oxygen partial pressure, blood perfusion and nutrients in the tumor microenvironment (TME) suggest that infiltrating immune cells in different sites experience different microenvironments with corresponding changes in the metabolic mode, that is, immune cell metabolism is heterogenous in the TME. Furthermore, the present review found that lipid metabolism can support the immunosuppressive microenvironment in breast cancer based on a review of published literature. Research in this field is still ongoing; however, it is vital to understand the metabolic patterns and effects of different microenvironments for antitumor therapy. Therefore, this review discusses the metabolic responses of various immune cells to different microenvironments in breast cancer and provides potentially meaningful insights for tumor immunotherapy.

Contents

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1. Introduction

Breast cancer is one of the most common malignant tumors in women (1). Research has demonstrated that breast cancer has certain immunogenicity (2). The types, degree and related phenotypes of immune cells infiltrating the tumor microenvironment (TME) are closely related to the prognosis of patients (3-5). However, almost all aspects of the immune response, such as the maturation and antigen presentation capacity of dendritic cells (DCs) and the infiltration and differentiation of immune cells, are closely related to the metabolic patterns of these cells and surrounding cells (6,7). The TME changes along a relatively smooth gradient, from a well-perfused perivascular area to an ischemic area, suggesting that immune cells will experience a variety of different microenvironments (8), which may vary from nutrient-rich, oxygen-sufficient sites to low-oxygen sites with competition for nutrients and even to hypoxic, nutrient-deprived sites or sites with metabolic waste accumulation (Fig. 1); this variation may also be a useful explanation for the early suppression of antitumor immunity. At the same time, it also leads to the metabolic heterogeneity of immune cells in the TME, which creates challenges for tumor immunotherapy.

Since Otto Warburg proposed in 1924 that tumor cells tend to produce energy rapidly through glycolysis (9), the important role of metabolic reprogramming in tumor development has gradually been recognized (10,11). However, the importance of lipid metabolism was generally ignored over the years, but lipid metabolism has been widely studied in the past few years. Based on previous research, we speculate that changes in lipid metabolism, especially fatty acid metabolism, are crucial in determining the immune activity or tolerance of immune cells. The metabolic pattern of infiltrating immune cells in breast cancer changes significantly across different environments. However, at present, research on this topic is not cohesive. Therefore, this review focuses on the metabolic changes

in several immune cells with relatively high infiltration in different microenvironments in breast cancer and addresses how cells are changed into a 'bystander' or an 'accomplice' by regulating lipid metabolism. It is helpful to further understand the metabolic heterogeneity of infiltrating immune cells in breast cancer in the contexts of different backgrounds and provide novel ideas for immunotherapy. Abbreviations used in the present review are included in Table I.

2. Relationship between metabolism, tumors and immune cells

To understand the metabolic heterogeneity of immune cells in breast cancer, the relationship among tumor cells, immune cells and metabolism was analyzed in this review. Every aspect of tumor tissue that differs from that of normal tissue may be the cause of tumor metabolic heterogeneity. In recent years, an increasing number of studies have shown that the metabolic patterns of different groups in tumors are coupled with each other through metabolism, and metabolism plays a very important role in the functional maintenance and directional differentiation of immune cells; that is, immune cells in the TME will withstand metabolic reprogramming, undergoing either activation to play an antitumor role or immune tolerance to promote tumor progression (12-15).

Metabolic heterogeneity is one of the markers of breast cancer, and the genetic and phenotypic diversity of breast cancer is the main obstacle when treating tumors (16). Due to random genetic changes, intratumoral heterogeneity is generally considered to be chaotic. By contrast, changes in tumor cell metabolism produces predictable extracellular metabolite gradients, which combined with the distance of the tumor cells from the blood supply (17), forms a unique TME and thus coordinates the diverse phenotypes of various cells (18,19). Previous studies have shown that breast cancer cells have a higher extracellular environment acidification capacity than normal breast cells (20,21). On the other hand, the rapid growth of cells is faster than the rate of capillary formation in cancer, leading to gradual hypoxia in breast cancer tissue (22,23), and creating a nutrient-deficient environment (24). The series of gradient changes described above leads to immune cells making metabolic adjustments that correspond to these different microenvironments. The present review described the changes in the metabolic patterns of tumor-infiltrating immune cells in different microenvironments and the induction of a tolerance phenotype by lipid metabolism.

3. Lymphocytes

Tumor-infiltrating lymphocytes (TILs) have a strong prognostic value in various types of cancer (25), and they are also the most common type of infiltrating immune cell in breast cancer (26). T cells comprise heterogeneous cell groups with a wide range of effector mechanisms, ranging from immunosuppression to cytotoxicity.

Metabolic characteristics of T lymphocyte subtypes. T lymphocytes are activated to become mature T lymphocytes that show associated functions via stimulation of T cell receptor (TCR) signaling. Activated T cells significantly upregulate glycolysis and lactate production (27). ADP-dependent glucokinase (ADPGK), which is a protein typically found in Archaea whose function in eukaryotes was unknown, is activated in this process, which is accompanied by rapid glucose uptake and decreased mitochondrial oxygen consumption, thus resulting in the increase of glycolysis flux (28). However, it cannot be ignored that mitochondria-dependent metabolism still plays an important role in the T cell response (29). In the absence of TCR stimulation, immature T cells remain in a dormant state, reducing the expression of nutrient transporters, but still maintaining certain catabolic processes, including autophagy, oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) (30). According to the literature, different lymphocyte subtypes exhibit different metabolic patterns during the activation process, in which the effector T lymphocytes show high glycolysis and lipogenesis (31), while regulatory T cells (Tregs) show higher lipolysis and lipid oxidation (32).

Influence of lactic acid and hypoxia on the T cell phenotype. Due to factors such as hypoxia, lactate and adenosine accumulation, nutrient deficiency, and immunosuppression, the activation and survival of T cells face great challenges. Both normal and tumor cells can adapt to hypoxia or a hypoxic microenvironment by regulating hypoxia inducible factor (HIF). A lack of oxygen supply, or hypoxia, will increase the expression and stability of HIF in T lymphocytes, thus activating certain molecular programs, including glycolysis (33). HIF mainly reduces oxygen consumption by increasing the expression of pyruvate dehydrogenase kinase 1 (PDK1), thus promoting T cell adaptation to hypoxia (34). At this time, T lymphocytes also play a corresponding role by regulating their own metabolic mode. However, in the central tumor area with extreme hypoxia, T cell survival is threatened. To survive, T cells reduce their dependence on glycolysis, developing an immunosuppressive phenotype (35). Studies have demonstrated that breast cancer tumors secreting a large amount of lactate have relatively great metastatic potential, and the prognosis of patients with these tumors is worse than that of patients with tumors secreting less lactate (36,37). One of the possible reasons is that the acidic environment formed by the accumulation of a large amount of lactate inhibits the proliferation and function of T cells (38,39). Activated T cells also depend on glycolysis. Due to the high demand for energy in the processes of proliferation and cytokine production, to ensure continuous glycolysis, cells pump out lactate molecules. The large accumulation of lactate in the breast cancer environment leads to an inappropriate lactate gradient between the extracellular environment and the cytoplasm, thus reducing energy metabolism and ultimately infiltrated T cells gradually polarize into the immunosuppressive Treg phenotype (40).

In addition, glucose deprivation increases the ability of T helper (Th) cells to secrete transforming growth factor β, thus confirming the shift of the microenvironment from immunostimulatory to immunosuppressive (41). It has been reported that T cells can transition into a metabolic mode to perform lactate uptake in a glucose-deficient TME (42). Besides, the reversal of the lactic dehydrogenase reaction to generate pyruvate depletes nicotinamide adenine dinucleotide and effectively inhibits GAPDH activity and glycolytic flux (43),

Table I. List of abbreviations in the review.

Abbreviation	Full name
2-DG	2-deoxyglucose
AMPK	Adenosine 5'-monophosphate (AMP) -activated protein kinase
ARG1	Arginase 1
ATP	Adenosine triphosphate
BMDCs	Bone marrow-derived dendritic cells
CCR7	C-C motif chemokine receptor 7
CD36	CD36 molecule
CD56	CD56 molecule
CD68	CD68 molecule
cDCs	Conventional dendritic cells
DCs	Dendritic cells
EMT	Epithelial-mesenchymal transition
FAO	Fatty acid oxidation
FASN	Fatty acid synthetase
FATP2	Fatty acid transporter 2
FATP4	Fatty acid transporter 4
IL-4	Interleukin-4
LDL	Low density lipoprotein
MDSCs	Myeloid-derived suppressor cells
mTOR	Mechanistic target of rapamycin kinase
NK cells	Natural killer cells
NO	Nitric oxide
OXPHOS	Oxidative phosphorylation
PMN-MDSCs	Polymorphonuclear myeloid-derived suppressor cells
Foxp3	Forkhead box P3
GLUT1 (SLC2A1)	Solute carrier family 2 member 1
HIF	Hypoxia inducible factor
IFN-γ	Interferon-γ
IL-12	Interleukin-12
IL-15	Interleukin-15
IL-2	Interleukin-2
TAM	Tumor-associated macrophage
TCR	T cell receptor
Th cells	T helper cells
TILs	Tumor-infiltrating lymphocytes
TME	Tumor microenvironment
Tregs	Regulatory T cells
VLDL	Very low-density lipoprotein

which is particularly harmful to cytotoxicity and effector T cells. Of note, immunosuppressive Tregs show resistance to lactate inhibition via downregulation of c-Myc expression by forkhead box P3 (Foxp3), which reduces glycolysis dependence (44). At the same time, T cells stimulated by TCR signaling in glutamine- and glucose-deficient conditions preferentially differentiate into Tregs, which may be because their oxidative phenotypes are metabolically suited for survival in this environment (36). When the survival of cells is threatened, their metabolic mode changes, and glucose is no longer the first-choice energy source; instead, cells are more inclined to use lipids for energy supply and maintenance (45). This is because the outer edge of tumor tissue with abundant blood vessels in breast cancer mostly contains effector T cells, and in the area lacking a sufficient blood supply, Tregs have more advantages; these differences reflect the metabolic heterogeneity of TILs (46).

Contribution of lipid metabolism to the differentiation of T cells into Tregs. Various studies have found that OXPHOS and glycolysis, especially glycolytic flux, are significantly increased in effector T cells (47-49). By contrast, Tregs show a unique metabolic program that mainly utilizes mitochondrial oxidation of lipids and pyruvate (50,51). Other evidence has suggested that inhibition of glycolysis obstructs the development of Th1 and Th17 cells, but promotes the production of Tregs (52). Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) is a major sensor and regulator of energy metabolism in mammalian cells. AMPK interferes with T cell differentiation and effector functions by suppressing mechanistic target of rapamycin kinase (mTOR) and subsequently inhibiting glycolysis and enhancing lipid oxidation (52).

Foxp3 is highly expressed in Tregs. Studies have reported that Foxp3 plays an important role in the regulation of metabolism (53,54). In a previous study, $CD4^+$ T cells were stimulated and transduced to express Foxp3 under neutral conditions, and the results showed that the expression of lipid metabolism-related genes was significantly increased in the Foxp3-expressing cells, while the expression of genes related to glucose and nucleic acid metabolism was downregulated (55). It is speculated that Foxp3 may directly regulate the PI3K-AKT-mTORC1 pathway or indirectly regulate the expression of metabolic genes and establish a phenotype of glycolysis inhibition (55). Immune cells respond to activation and toll-like receptor (TLR) signaling by increasing solute carrier family 2 member 1 (GLUT1) expression and glycolysis (55). By contrast, Foxp3 reduces GLUT1 expression, glycolysis and anabolism, indicating an activated mitochondrial oxidation pathway (56). *In vitro* studies have also demonstrated that $Foxp3^+$ Tregs mainly rely on lipid oxidation to promote mitochondrial OXPHOS, and it has been speculated that Foxp3 expression is the basis of this metabolic preference (55).

4. Macrophages

Tumor-associated macrophages (TAMs), another 'main force' in the TME, have been observed in the invasive front of breast cancer tumors in patients (57). Previous reports demonstrated that compared with malignant cells that have not undergone epithelial-mesenchymal transition (EMT), breast cancer cells with EMT changes have the ability to polarize macrophages into the M2 phenotype, suggesting that macrophages in the breast cancer microenvironment play an important role in tumor invasion (58,59). As commonly known, the main subtypes of macrophages are proinflammatory M1 macrophages and anti-inflammatory M2 macrophages. M1 macrophages mainly secrete cytokines such as interferon-γ (IFN-γ), interleukin (IL)-8 and TNF-α, which play pro-inflammatory and

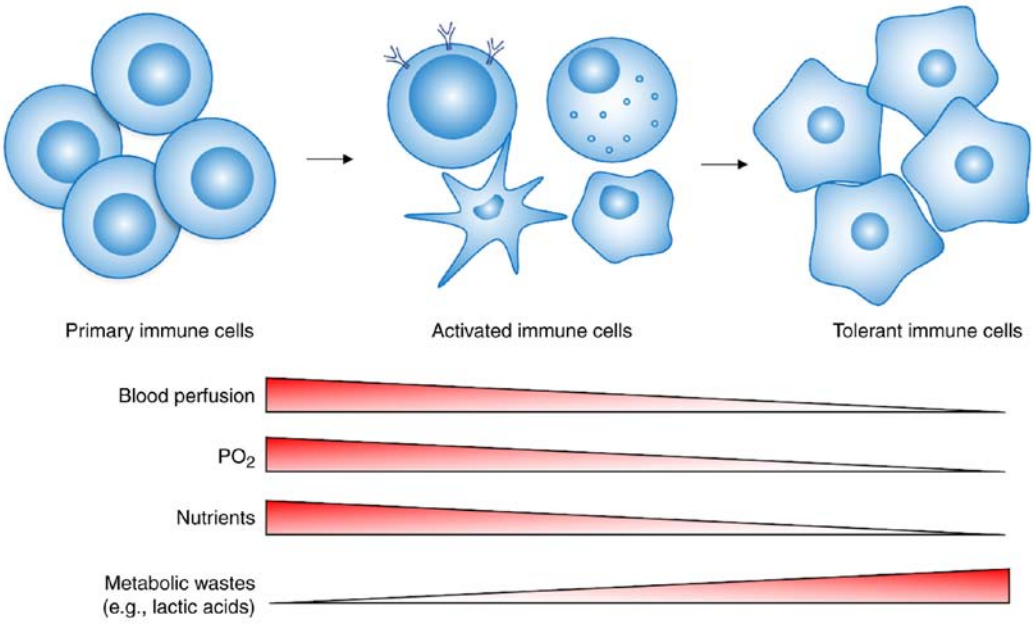

Figure 1. Tumor-infiltrating immune cells experience a complicated microenvironmental challenge. The tumor microenvironment transitions from areas with abundant nutrients and oxygen to those with low oxygen and competition for nutrients or even hypoxia, nutrient deprivation and metabolic waste accumulation, and the state of immune cells changes accordingly. Immune cells are activated from the initial state to become immune cells with different effector functions. When survival is threatened, immune cells develop a resting or tolerant phenotype.

antitumor roles. On the other hand, M2 macrophages mainly secrete factors such as IL-13, C-C motif chemokine (CCL)17 and CCL18 to promote tumor development (60,61). Due to a combination of numerous factors and the complexity of the TME, the phenotype of TAMs may be between M1 and M2 types, or different from M1 or M2 types that can't be regarded as either type specifically. Thus, TAMs can no longer be simply considered either/or populations (62).

Metabolic characteristics of macrophage subtypes. To clarify the metabolic characteristics of macrophage subtypes, cells can still be divided into M1 and M2 type macrophages. M1 macrophages show enhanced aerobic glycolysis, increased pentose phosphate pathway activity and fatty acid synthesis flux. However, at the level of succinate dehydrogenase and isocitrate dehydrogenase, M1 macrophages also exhibit incomplete OXPHOS, and mitochondrial adenosine triphosphate (ATP) synthesis is blocked (63). M2 macrophages break down arginine into urea and urethane via arginase 1 (ARG1). ARG1 is a representative marker of M2 macrophages, and nitric oxide (NO) production in M2 macrophages is blocked, resulting in inhibition of nitroso-mediated OXPHOS, which is conducive to maintaining the M2 phenotype (64). M2 macrophages show relatively low levels of glycolysis and enhanced FAO to fuel OXPHOS (65). Highly glycolytic tumor cells may prevent polarization into the M1 phenotype by inducing glucose deprivation, while the abundance of fatty acids may affect the differentiation of cells into the M2 phenotype (66,67).

Influence of lactic acid and hypoxia on the macrophage phenotype. Similar to TILs, tumor-infiltrating macrophages with different spatial distributions face different challenges and respond accordingly. Carmona-Fontaine *et al* (19) found that TAMs expressing ARG1 were almost completely located in the ischemic tumor area, while TAMs expressing mannose receptor C-type 1 (MRC1) were found in the perivascular and other well-nourished tumor areas, and the research also showed that the subgroup of TAMs expressing MRC1 in the perivascular region of patients with breast cancer was important for tumor recurrence after chemotherapy (19). Some studies have reported that lactate produced by breast cancer cells, a key metabolite in the TME, can promote M2-like polarization of macrophages by inducing high expression of VEGF and ARG1 in macrophages, and this series of changes may be mediated by HIF-1α (68,69). Almost all studies have provided extensive evidence of the synergistic effect of hypoxia and lactate (70,71). When macrophages in normoxic or hypoxic environments are treated with various lactate doses, the ARG1 protein level in macrophages increases in hypoxic conditions, but not in normoxic conditions (19). Additionally, macrophages activated by lactate and/or hypoxia can induce aerobic glycolysis and epithelial stromal transformation in tumor cells by regulating the CCL5/C-C chemokine receptor type 5 (CCR5) axis, forming a regulatory feedback loop to promote the progression of breast cancer (72). The metabolic pattern of M1 macrophages is similar to that of tumor cells, showing highly activated glycolysis, which indicates that M1 macrophages and tumor cells compete with and suppress each other (73). By contrast, M2 macrophages preferentially use FAO, which is more conducive to their survival in the TME, and became a favorable promoter of tumor progression (74).

Important role of lipid metabolism in the immunosuppressive TAM phenotype. TAMs promote tumor growth and metastasis by inhibiting tumor immune surveillance. There is evidence that the immunosuppressive phenotype of TAMs is regulated by long-chain fatty acid metabolism, especially unsaturated fatty acid metabolism (75). *In vitro*, the addition of unsaturated

fatty acids was found to polarize myeloid cells derived from the bone marrow into M2 macrophages with a strong inhibitory ability. Lipid droplets play a vital role by regulating the catabolism of free fatty acids during mitochondrial respiration (76). IL-4-induced M2 macrophages increase their expression of CD36, thus enhancing the uptake of very low-density lipoprotein (VLDL) and LDL, activate FAO, and rely on FAO to support proliferation (77). Inhibition of mTOR eliminates the mitochondrial respiration induced by lipid droplets, thus eliminating the immunosuppressive effect of TAMs (75). A previous study reported that simvastatin repolarizes TAMs and promotes M2 to M1 phenotypic conversion through cholesterol-related liver X receptor/ATP binding cassette transporter A1 regulation (78). These results suggested that lipid metabolism plays an important role in the differentiation and functional maintenance of M2 macrophages and that further study of lipid metabolism has the potential to identify potential targets and generate novel antitumor treatments.

5. Natural killer (NK) cells

NK cells are key components in innate immunity. They are mainly generated from hematopoietic stem cells that develop in the bone marrow and distribute to multiple peripheral tissues after maturation (79). They have the potential to kill tumor cells in different ways without prior sensitization, so they have become an important tool in cancer immunotherapy (80).

Metabolic characteristics of NK cells. In general, resting NK cells use OXPHOS to meet their own steady-state needs because this pathway can effectively generate energy without requiring an excessive investment in synthesis (81). When NK cells are activated, they change their metabolic mode to be able to create the large number of biosynthetic precursors required for the synthesis of effector molecules. NK cells have been proven to be able to strongly upregulate glycolysis and the OXPHOS pathway (82,83). Activated NK cells transform glycolysis-derived NADH into mitochondrial NADH via the citrate-malate shuttle mechanism, which promotes OXPHOS and the synthesis of ATP (84). NK cells are widely characterized as $CD56^{dim}$ and $CD56^{bright}$ (85). These subsets also differ in terms of metabolism. $CD56^{bright}$ cells are more sensitive to metabolic changes, and their upregulation of the expression of centralized metabolic markers is stronger than that of $CD56^{dim}$ cells (82). Schafer *et al* (86) recently reported that NK cells with low reactivity used mitochondrial respiration to activate cytotoxic function, while functional NK cells showed increased glycolysis accompanied by OXPHOS. One of the main limitations of NK cell activity is the immunosuppressive TME. Tumors and other immune cells create conditions that favor tumor proliferation, while also blocking the activation of NK cells.

Influence of lactic acid and hypoxia on the NK cell phenotype. The activity of NK cells is lower near the ischemic area in the tumor center, and it is difficult for even NK cells to infiltrate into the central area. The lack of nutrients and oxygen, and high concentrations of tumor-derived metabolites (such as lactate and adenosine) disrupt the metabolism of NK cells in the TME (80). Short-term exposure to hypoxia enhances the function of NK cells (87), but after long-term hypoxia, NK cells upregulate HIF-1α expression, resulting in a change in the transcriptional profile and obvious downregulation of the expression of cytotoxic receptors, such as natural killer cell p30-related protein (NKp30), NKp44, NKp46 and natural killer group 2 member D (88). At the same time, HIF-1α also affects the following: i) Glycolytic enzymes M2 isoform of pyruvate kinase and phosphoglycerate kinase 1; ii) the metabolite transporters, including GLUT1, solute carrier family 2 member 3, solute carrier family 1 member 5 and solute carrier family 16 member 4; and iii) enzymes involved in biosynthesis, such as fatty acid synthetase (FASN) and glucose 6-phosphatedehydrogenase (89). The expression of IFN-γ in mature NK cells is decreased, the production of IFN-γ is decreased, and OXPHOS is reduced (90).

A recent study showed that adenosine attenuated the metabolic activity of IL-12/15-stimulated human NK cells by inhibiting OXPHOS and glycolysis (91). Furthermore, it has also been demonstrated that the uptake of lactic acid by NK cells in the TME leads to intracellular acidification and energy metabolism disruption (92). On the one hand, the increasing demand of tumors for amino acids and the lack of a fuel supply in the microenvironment reduces the functions of NK cells. We also speculated that the consumption of amino acids by tumor cells and tumor-related cells will lead to the accumulation of immunosuppressive metabolites in the TME, which indirectly affects the function of NK cells. For example, myeloid-derived suppressor cells (MDSCs) upregulate the expression of arginase and inducible NO synthetase, which use arginine as a substrate, and the latter metabolizes arginine into NO. It has been found that NO weakens the cytotoxicity of antibody-dependent NK cells (93). NK cells in breast cancer gradually experience an increasingly harsh environment. The metabolism of NK cells is negatively affected, with the cells transitioning from an antitumor function to being unable to undergo activation or even becoming unable to survive in the central tumor area without blood perfusion (94).

Effect of lipid metabolism activation on NK cell immune tolerance. NK cells are significantly inhibited in the severe TME and are even found in the resting state (95). It was found that genes related to glycolysis and OXPHOS were downregulated in NK cells undergoing severe TME. By contrast, the activity of lipid metabolism is increased (96,97). Studies have also confirmed that when NK cells survival is threatened, lipid metabolism becomes the preferred mode of metabolism (98,99). In a mouse model of breast cancer, NK cells appeared to accumulate lipids *in vivo*, which was mediated by CD36 and CD68, after surgery. These NK cells showed an inhibitory effector function with downregulation of the expression of perforin- and granzyme-related genes (100). Peroxisome proliferator-activated receptor drives lipid accumulation in NK cells, leading to complete 'paralysis' of cell metabolism and transportation (101). Preventing lipids from entering the mitochondria reverses NK cell metabolic paralysis and restores cytotoxicity (101). Compared with immune cells with an immunotolerant phenotype, NK cells have shown a smaller effect on cell function mediated by lipid reprogramming, and the mechanism of action is not sufficiently understood (101). However, it is of great significance and value

to identify the potential targets of lipid metabolism in NK cells and thereby improve immunotherapy.

6. DCs

Tumor-associated DCs have major defects in function and activity, and promote tumor immunosuppression. Studies have reported that abnormal lipid accumulation in DCs is one of the main mechanisms leading to DC dysfunction (102,103). DCs are important regulators of activation or tolerance in the adaptive immune response (104), and are also one of the most important types of antigen-presenting cells in the breast cancer microenvironment (105). DCs can not only regulate immunogenicity, but also induce tolerance. One of the key factors determining this functional fate is the metabolic process of DCs (13).

Metabolic characteristics of DC subtypes. In the process of DC activation, glycolysis and glucose, as the preferred carbon source, can promote an immunogenic or inflammatory state, while OXPHOS and FAO are conducive to the transformation of tolerant DCs (106,107). Glycolysis is important for the maturation and function of both conventional DCs (cDCs) and bone marrow-derived DCs (BMDCs). Treatment with 2-deoxyglucose (2-DG, an inhibitor of glycolysis) impairs the expression of costimulatory markers, the production of IL-12, and functioning by BMDCs and cDCs (108). Glucose can promote the migration of BMDCs and cDCs along a C-C motif chemokine ligand 21 gradient, which can be inhibited by 2-DG treatment (108). In addition, glycolysis is also needed to maintain the slender cell shape of BMDCs and promote CCR7 oligomerization so that DCs can move and migrate to the draining lymph nodes (109).

Influence of lactic acid and hypoxia on the DC phenotype. The duality of DC immunoregulatory functions mainly depend on the differentiation and activation state of DCs (104). DCs undergo metabolic transformation in the process of maturation, from FAO and OXPHOS to glycolysis. Unlike the transformations of tumor cells and effector T cells, this transformation of DCs does not promote cell division, but is crucial in the activation and survival of DCs after TLR stimulation (110). The concentration of lactate after glycolysis, rather than the availability of oxygen tends to shift the differentiation of DCs in the direction of tolerance (111). The high concentration of extracellular lactate in the TME may prevent lactate output from glycolysis-dependent DCs. Tumor-derived lactate is an important factor regulating the DC phenotype in the TME, which may play a key role in the tumor escape mechanism (112). Additionally, the effect of hypoxia on DCs is very important in regulating the quality and intensity of the immune response. DCs derived from human monocytes exposed to hypoxia express high levels of HIF-1α. Short-term hypoxia can indeed enhance the migration of DCs through a HIF-1α-mediated glycolytic pathway, thus showing obvious immunogenicity. However, long-term hypoxia can cause cell death (113).

Of note, recent evidence has shown that the role of glucose in DCs depends on the state of adjacent cells (108). For example, glucose can promote the immune function of DCs, but this function is severely inhibited in areas of high ischemia and hypoxia in tumors (114). The reason is that the high rate of glycolysis of tumor cells leads to local glucose deprivation, and thus the substrate of glycolysis may not be used by DCs. Therefore, the TME may not be suitable for activation of DCs dependent on glycolysis, and thus DCs may be required to transition to fatty acid-dependent oxidation (8).

Important contribution of lipid metabolism to tolerant DCs. Herber *et al* (102) evaluated lipid accumulation in tumor-infiltrating DCs that would hinder antigen presentation and major histocompatibility complex II expression, and inhibition of acetyl-CoA carboxylase 1 reversed the effects of lipids, suggesting that this process involved the fatty acid biosynthesis pathway (102,115). FASN is a key enzyme in the *de novo* synthesis of fatty acids. It directly supports the abilities of proliferation and metastasis in tumor cells (116,117). A study reported that FASN was highly expressed in ovarian cancer cells, which led to lipid accumulation in the TME that inhibited the ability of tumor-infiltrated DCs to support antitumor T cells, resulting in antigen presentation that led to T cell activation defects (118).

DCs isolated from various tumor models and patients with tumors have shown that the accumulation of lipids in DCs limits the cross presentation of antigens (102). Consistent with these findings, the accumulation of lipid droplets prevents DCs from inducing antitumor T cell responses (119). DCs with an immunotolerant phenotype show strong activation of endoplasmic reticulum stress and X-box binding protein 1 spliced by endoplasmic reticulum stress response factor, which induces the biosynthesis of triglycerides and leads to abnormal lipid accumulation (103). The aforementioned studies suggest that lipid metabolism plays an important role in the development of tolerance in DCs.

7. Other immune cells

There are numerous types of infiltrated immune cells in the TME. In addition to the main immune cells mentioned above, there are also some immune cells that are less common, but still play important roles in antitumor immunity, such as mast cells, monocytes, eosinophils and basophils (120,121). At present, the metabolic reprogramming of these cells in the TME is relatively unstudied. Some of these cell types have been studied briefly, while there are a few that have not yet been reported on, but they are worthy of further exploration. Mast cells are unique tissue-resident immune cells that can secrete a variety of bioactive compounds, which can stimulate, regulate or inhibit the immune response. Increasing evidence has reported that mast cells infiltrate breast cancer, but whether they are a driving force or have an opposing role in breast cancer progression is controversial (122-124).

In breast cancer, myeloid cells can transform into MDSCs and play a key role in tumor immunosuppression. Decades ago, studies demonstrated that cancer cells rather than stromal cells prefer to export fatty acids to form fatty acid-rich niches (125,126). MDSCs absorb fatty acids from the TME and use these molecules in a variety of ways to maximize their effects. An increase in fatty acid uptake by MDSCs leads to the accumulation of intracellular lipids, and lipid-overloaded MDSCs have a stronger immunosuppressive effect on $CD8^+$ T cells (127). Cao *et al* (128) revealed that the expression of fatty acid transporter 4 (FATP4) was significantly upregulated in MDSCs. Other studies have also

Table II. Status of tumor-infiltrating immune cells under different metabolic patterns.

Immune cells	Main metabolic modes	States
T lymphocytes	Reduced nutrient transport and maintained catabolism	Primary
	Glycolysis and lactic acid production	Activated
	Lipolysis and lipid oxidation	Immunosuppressive
Macrophages	Enhanced aerobic glycolysis, pentose phosphate pathway activity and fatty acid synthesis	M1
	Low levels of glycolysis and enhanced fatty acid oxidation	M2
NK cells	OXPHOS	Resting
	Upregulated glycolysis, OXPHOS and lipid synthesis	Activated
	Lipid metabolism	Inactivated
Dendritic cells	Glycolysis	Immunostimulatory
	OXPHOS and fatty acid oxidation	Immunosuppressive

OXPHOS, oxidative phosphorylation.

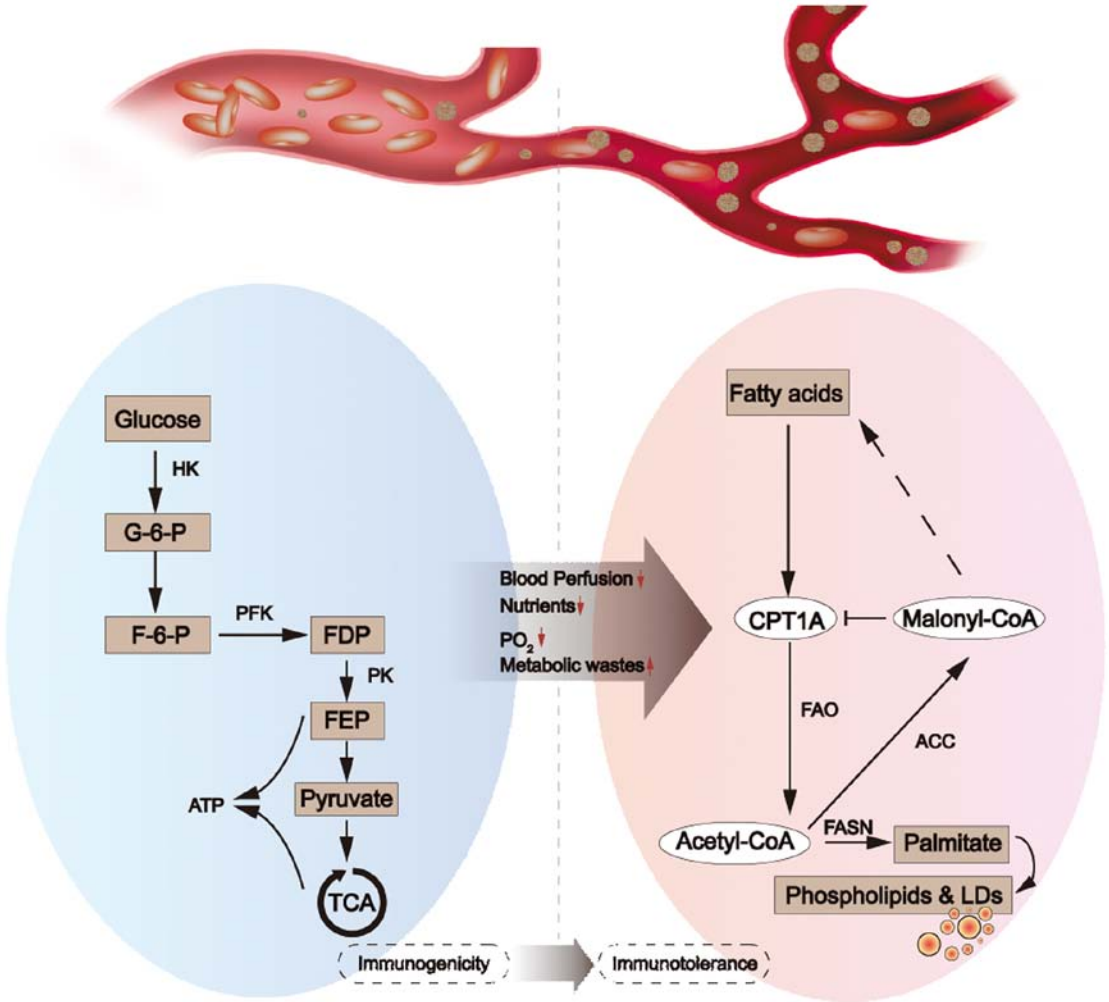

Figure 2. Priority of the immune cell metabolic mode is lipid metabolism during the transition from effector function to immune tolerance in the tumor microenvironment. Immune cells show corresponding effector functions with the metabolic mode of glycolysis. Glucose generates pyruvate through a series of reactions performed by the related enzymes, including HK, PFK and PK, and rapidly produces ATP and intermediate metabolites (G-6-P, F-6-P, FDP and PEP). However, immune cells are challenged by an environment comprising insufficient blood supply, decreased oxygen partial pressure, lack of nutrients and increased metabolic waste, which gradually leads to a tolerance phenotype, and the metabolic mode favors lipid metabolism, including FAO, and lipid synthesis (CPT1A, ACC, FASN, triglyceride and cholesterol). LDs play an important role in this process. On the one hand, LDs store excess lipids; on the other hand, LDs provide energy when needed. HK, hexokinase; PFK, phosphofructokinase; PK, pyruvate kinase; G-6-P, glucose-6-phosphate; F-6-P, fructose 6-phosphate; FDP, fructose 1,6-bisphosphate; PEP, phosphoenolpyruvate; FAO, fatty acid oxidation; CPT1A, carnitine palmitoyl transferase 1A; ACC, acetyl CoA carboxylase; FASN, fatty acid synthase; LDs, lipid droplets; TCA, trichloroacetic acid.

reported that the upregulation of FATP2 expression in polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) is the key regulator of PMN-MDSC immunosuppressive function. FATP2 promotes the accumulation of arachidonic acid, resulting in the synthesis of prostaglandin E2 in MDSCs, which enhances MDSC immunosuppressive activity (129). A series of changes lead to MDSCs surviving in the harsh TME. The role of lipid metabolism in determining whether immune cells differentiate into an immunotolerant phenotype has been ignored, as has whether immunosuppressive cell infiltration of tumor tissue is induced by a harsh environment, which provide novel ideas and insights for tumor immune research.

8. Conclusions

The complex and dynamic changes in the TME cause infiltrating immune cells to face different challenges. Compared with the randomness and chaos of genetic changes, predictable extracellular metabolite gradients allow speculation on the metabolic changes experienced by immune cells. By studying and summarizing the changes in the metabolic patterns of several typical tumor-infiltrating immune cell types in different TMEs (Table II), the present review concluded that mild hypoxia and a low lactic acid concentration are beneficial for immune cell activation and a metabolic mode favoring glycolysis. In such a scenario, the immune cells have certain immunogenicity and play an antitumor role. However, facing severe survival challenges, such as hypoxia, an extreme lack of nutrients and high acidity in the surrounding environment, the immune cells will gradually shift their metabolism mode into lipid metabolism, in some cases, even depending on FAO, and the immune cells will show a tolerant or inhibited phenotype (Fig. 2). The function of immune cells also changes when the metabolism mode changes, which provides us with a number of potential immunotherapy targets and novel treatment ideas. If the key genes and enzymes of lipid metabolism can be targeted, it may be possible to reverse the tolerant phenotype of immune cells and enhance the antitumor effect. In the future, it is necessary to study the metabolic heterogeneity of immune cells and the causes of this heterogeneity, in order to provide more effective methods for tumor immunotherapy.

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Availability of data and materials

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Authors' contributions

HC, YS, ZY, SY, YL, MT, JZ and FZ contributed to the conceptualization, literature review, original draft preparation, editing and review of this manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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