

CD147-mediated reprogrammed glycolytic metabolism potentially induces immune escape in the tumor microenvironment (Review)

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Abstract. Impaired antitumor immunity or induced immunosuppression in the tumor microenvironment contributes significantly to tumor progression and resistance to immunotherapy. It is becoming increasingly recognized that dynamic metabolic programming orchestrates appropriate immune responses, whereas incorrect metabolic reprogramming

may underlie aberrant immune remodeling. Furthermore, pathways that control cellular metabolism and immune cell function by transcriptional and post-transcriptional mechanisms are intimately interlinked, including hypoxia-inducible factor 1 α , c-Myc and phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin signaling. Immunometabolism is an emerging research field involving investigation of the interaction between immunological and metabolic processes. It is likely that high levels of nutrient competition and metabolic interplay exist between tumor cells and infiltrating immune cells in the local tumor milieu, which consequently leads to a reduction in antitumor immunity or immune cell dysfunction. Recently, a metabolic molecular mechanism responsible for the tumorigenic capacity of cluster of differentiation (CD)147, which exhibits high expression on the surface of various malignant tumor cells and is associated with tumor progression via multiple non-metabolic molecular mechanisms, was identified. The aim of the present review was to focus on the glycolytic mechanism mediated by the upregulation of CD147 in tumors and tumor-imposed metabolic restrictions on tumor-infiltrating immune cells, and the consequent immunological hyporesponsiveness. Cellular metabolism is becoming increasingly acknowledged as a key regulator of T-cell function, specification and fate, and the manipulation of metabolic programming may elucidate therapeutic options for immunological disorders and tumor immunotherapy.

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Abbreviations: Akt/PKB, protein kinase B; AMPK, AMP-activated protein kinase; CD147, cluster of differentiation 147; CTLs, cytotoxic T lymphocytes; eIF-4E, eukaryotic translation initiation factor 4E; EMT, epithelial-to-mesenchymal transition; GLUT1/2/4, glucose transporter 1/2/4; GSK-3 β , glycogen synthase kinase-3 β ; HCC, hepatocellular carcinoma; HIF, hypoxia-inducible factor; HK2, hexokinase type 2; HRE, hypoxia-response element; LDHA, lactate dehydrogenase type A; MCT1/4, monocarboxylate transporter 1/4; MDM2, mouse double minute 2 homolog; MDSCs, myeloid-derived suppressor cells; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; P53, protein 53; P70S6K, ribosomal protein S6 kinase; PD-L1, programmed cell death ligand-1; PFK1/2, phosphofructokinase1/2; PFKM, phosphofructokinase; PKM2, pyruvate kinase muscle isoform 2; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog; Rheb, Ras homolog enriched in brain; Slug, zinc-finger transcriptional factor Slug; Snail, zinc-finger transcriptional factor Snail; SP1, specificity protein 1; TCF4, T-cell factor 4; TGF- β , transforming growth factor- β ; TIGAR, TP53-induced glycolysis and apoptosis regulator; TILs, tumor-infiltrating lymphocytes; TSC1/TSC2, tuberous sclerosis complex1/2

Key words: aerobic glycolysis, tumor immune escape, metabolic manipulation

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1. Introduction

The two emerging hallmarks of metabolic reprogramming and evasion of immune destruction represent significant conceptual progress in the field of tumor research (1). Tumor cells preferentially uptake and utilize glucose via aerobic glycolysis, even in the presence of sufficient oxygen to support the mitochondrial oxidative respiration, a phenomenon referred to as the 'Warburg effect' (2). The role of the immune system in tumor cell recognition and elimination is becoming increasingly ambiguous, as tumor cells have developed several mechanisms to avoid immune responses (3-5). The functional impairment of effector T cells (Teffs) and the induction of immunosuppressive T cells in the tumor microenvironment may lead to immune escape or evasion by cancer cells (6-8). The mechanisms responsible for T-cell dysfunction or hyporesponsiveness are complicated (9), and the association between tumor metabolic switch and immune tolerance has been attracting increasing attention (1,10-13).

The immune functions of Teffs are intimately associated with their metabolic regulation (14-18). Clonal expansion and antitumor function acquisition of T cells upon activation, which are energy-demanding processes, are accompanied by a marked shift in metabolism from energy-oriented catabolic oxidation in naive T cells to biosynthetic aerobic glycolysis in activated T cells (14). The metabolic and immunological functions of Teffs may be impaired by tumor cells due to the tumor-imposed nutrient depletion and accumulation of tumor-derived immunomodulatory metabolic intermediates, including lactate, in the tumor microenvironment (11-13). The manipulation of metabolic reprogramming in T cells is currently considered as a potential therapeutic target to regulate the antitumor function and fate of Teffs (19,20).

Although several intracellular signaling pathway molecules in the metabolic reprogramming of tumor cells and Teffs have been identified, including hypoxia-inducible factor-1 (HIF-1), c-Myc and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) (15,21-26), how tumor cells cope with extracellular metabolic signals and transduce extracellular signals to intracellular stimuli remain to be fully elucidated. CD147, also referred to as HAB18G/CD147 in humans, is a transmembrane protein that has been reported to be overexpressed on the surface of various types of malignant tumor cells (27,28). Upregulation of the expression of CD147 has been found to contribute significantly to malignant potential and poor prognosis through triggering the production and release of extracellular matrix metalloproteinase (MMP) and vascular endothelial growth factor (29-33). An increased dependence on aerobic glycolysis inevitably results in an increased production of lactic acid, and this surplus of lactic acid has to be exported in order to prevent cellular acidosis and maintain cellular homeostasis (34). Monocarboxylate transporters (MCTs) catalyze the transport of monocarboxylates, including L-lactate, across plasma membranes (35,36). CD147 has also been described as a chaperone assisting in the

folding, stability, membrane expression and functionality of MCTs (37), suggesting the involvement of CD147 in metabolic regulation (34). The aim of the present review was to highlight the immunosuppression in the tumor microenvironment induced by underlying glycolytic mechanism reprogramming and discuss the therapeutic potential of targeted metabolic manipulation in tumor immunotherapy.

2. Glycolytic metabolism of tumor-infiltrating immune cells and underlying regulatory signaling pathways

An emerging theme in immunology is that metabolic adaption and lymphocyte function are intimately linked, and changes in cellular metabolism have been shown to be associated with altered immunological function (18). Tumor cells have been the focus of investigations on the metabolic switch, although the metabolic reprogramming of immune cells, particularly tumor-infiltrating lymphocytes (TILs), has not been investigated as extensively. Warburg was also one of the first to examine leukocyte metabolism, and found that leukocyte stimulation led to a shift towards aerobic glycolysis from oxidative phosphorylation (OXPHOS), which is primarily used by resting leukocytes (38).

Distinct glycolytic metabolic programs are used by different immune cell subsets. Resting or naive T cells predominantly oxidize glucose-derived pyruvate, in addition to lipids and amino acids, via OXPHOS, to maintain quiescence and immune surveillance (39). Upon T cell activation, lipid oxidation is sharply reduced, and the cells rely instead on increased aerobic glycolysis to support extensive proliferation and Teff differentiation and function (14,40). At the end of an immune response, a small population of antigen-specific T cells survives to become long-lived memory T cells, which revert back to a metabolic program comparable with that of resting T cells (14,18,41). However, memory T cells exhibit an increased capacity for efficient energy generation, characterized by an increase in mitochondrial mass and, consequently, maximal mitochondrial spare respiratory capacity, which allows for the rapid and vigorous production of ATP upon repeat encounter with antigens (42,43). In addition to Teffs, activated T cells can differentiate into regulatory T cells (Tregs), which serve a critical role in self-tolerance and immunosuppression (3,44,45). Unlike Teffs, Tregs primarily use glucose-derived pyruvate and fatty acids to efficiently produce ATP through the tricarboxylic acid cycle and lipid β -oxidation (46). Distinct metabolic programs are required for functionally different T-cell lineage differentiation and commitment (47,48). A comprehensive study by Michalek *et al* demonstrated that Teffs, including T helper (Th)1, Th2 and Th17 cells, were selectively increased in glucose transporter (GLUT)1 transgenic mice, and were dependent on a highly glycolytic metabolism (48). Tregs, by contrast, expressed a low level of GLUT1 and relied on high rates of lipid oxidation (48). Gene array analysis on CD8⁺ cytotoxic T cells under conditions of glucose deprivation or incubated in the presence or absence of 2-deoxy-D-glucose, which inhibits glycolysis, demonstrated that multiple key gene expression events and effector functions were selectively inhibited, including the production of interferon- γ (IFN- γ), cell cycle progression and cytolytic

activity (49). Consistently, impaired T-cell metabolism directly contributed to T-cell dysfunction and exhaustion in leukemia, whereas the genetically increased expression of GLUT1 and hexokinase 2 (HK2) may partially restore T-cell function (50). The upregulation of glycolysis by the transgenic overexpression of GLUT1 or glycolytic genes was sufficient to augment T-cell activation, ultimately resulting in lymphadenopathy and a systemic lupus erythematosus-like autoimmunity in aging mice (17).

c-Myc and HIF-1 α signaling pathways regulate the glycolytic metabolism of immune cells. The identification of transcription factors potentially responsible for the metabolic reprogramming upon T-cell activation revealed c-Myc and HIF-1 α as two of the top-ranked candidates, as both were found to be induced at the mRNA and protein levels upon T-cell stimulation (21). c-Myc specifically upregulates the expression of all glycolytic genes, including GLUT1, lactate dehydrogenase type A (LDHA), HK2 and pyruvate kinase muscle isoform 2 (PKM2). Subsequently, the acute genetic deletion of c-Myc markedly inhibits the upregulated glycolytic activity. In addition, an HIF-1 α -mediated glycolytic switch regulates the balance of Th17/Treg differentiation (22,51). Th17- but not Treg-polarizing conditions elicited a HIF-1 α -dependent acceleration of glycolysis via upregulation of glycolytic enzyme expression. By contrast, the inhibition of glycolytic metabolism resulted in the inhibition of Th17 differentiation and promotion of Treg development. Upon investigation of the underlying molecular mechanism, HIF-1 α was found to be selectively induced in Th17 differentiation through the mammalian target of rapamycin (mTOR) signaling pathway, whereas the deficiency of HIF-1 α led to decreased Th17 commitment but enhanced generation of Treg, which protected mice from experimental autoimmune encephalomyelitis (22).

Role of PI3K/Akt/mTOR signaling in the metabolism of T cells. PI3K/Akt is activated by various stimuli in T lymphocytes, including T cell antigen receptor, costimulatory molecules, cytokine receptors and chemokine receptors (23,24,52), and PI3K/Akt signaling serves a fundamental role in T-cell activity. For example, the trafficking of GLUT1 to the cell surface and prevention of internalization in T cells are promoted by Akt (53). Of note, mTOR, as a downstream target of Akt, is activated by Akt and serves a key role in linking the activation of PI3K to Th-cell differentiation (54,55). mTOR is a catalytic unit of two distinct multi-protein assemblies, referred to as mTOR complex (mTORC)1 and mTORC2. The activation of mTORC1 can initiate a signaling cascade, which leads to metabolic reprogramming characterized by increased aerobic glycolysis. Of note, mTOR differentially regulates Treg and Th17 lineage commitments through the activation of specific signal transducer and activator of transcription pathways and, consequently, the induction of lineage-specific transcription factors (54). By contrast, rapamycin treatment, which targets mTORC1, has been shown to exhibit an inhibitory effect on glycolytic switching upon T-cell activation (56). AMP-activated protein kinase (AMPK), as a well-known evolutionarily conserved energy sensor, is activated by an increased AMP/ATP ratio and acts in opposition to mTORC1 to maximize energy production via promoting mitochondrial

phosphorylation (57). AMPK α 1^{-/-} T cells exhibit an impaired ability to transit from an anabolic and glycolytic metabolism to a catabolic and lipid oxidative state under metabolic stress (58).

3. Upregulated glycolytic metabolism in tumors and underlying molecular mechanisms

It is well established that malignant transformation is associated with a disrupted balance between oncogenes and tumor suppressor genes. From a metabolic perspective, this is associated with a reprogrammed metabolism and constitutes a molecular basis for the accelerated aerobic glycolysis in tumors (59-62).

Role of c-Myc in upregulated glycolytic metabolism in tumors. Accumulating evidence has confirmed that the MYC oncogene, the PI3Ks/Akt/ mTOR pathway and HIF-1 (62,63), in addition to tumor suppressor p53, are implicated in the metabolic reprogramming of tumor cells (64-66) (Fig. 1). The MYC oncogene encodes a transcription factor, c-Myc, which links altered cellular metabolism to tumorigenesis (67). Generally, c-Myc directly and/or indirectly regulates the expression of genes involved in glucose, glutamine and nucleotide metabolism. For example, glycolytic genes, including LDHA are directly upregulated by c-Myc (68); however, c-Myc can repress the expression of microRNA-23a/b to indirectly promote the protein expression of glutaminase and metabolism of glutamine (69). The depletion of c-Myc has been shown to result in the repression of several genes encoding enzymes rate-limiting for deoxyribonucleoside triphosphates (dNTPs) metabolism, including thymidylate synthase, inosine monophosphate dehydrogenase 2 and phosphoribosyl pyrophosphate synthetase 2. The depletion of c-Myc also leads to a decrease in dNTPs and inhibited cell proliferation (70). A number of glycolytic genes have been documented to be directly regulated by c-Myc in screens for c-Myc target genes, including GLUT1, HK2 and muscle phosphofructokinase (71,72). In addition, c-Myc may cooperatively serve a pivotal role in hypoxic adaptation with HIF-1 through upregulating pyruvate dehydrogenase kinase 1 under non-normoxic conditions, thereby accelerating glycolytic metabolism by favoring the conversion of pyruvate to lactate and suppressing mitochondrial oxidative respiration (72-74).

Role of PI3K/Akt/mTOR signaling in glycolytic adaption in tumors. The PI3K/Akt/mTOR signaling pathway has been found to be activated at a high level and contribute to the metabolic transformation of tumors (75,76) (Fig. 1). Akt, a serine/threonine kinase, has been shown to be constitutively activated in tumor cells through the amplification of PI3K, which phosphorylates membrane-associated phosphatidylinositol 4,5-bisphosphate (PIP2) to generate phosphatidylinositol 3,4,5-trisphosphate as an upstream activator of Akt (77). Human glioblastoma cells with constitutive Akt activity exhibit high rates of aerobic glycolysis through the direct effect of Akt on glucose metabolism, including upregulating the expression and/or localization of glucose transporters and glycolytic enzymes, including GLUT1 and HK2 (75). Akt also activates mTOR, which also contributes to the glucose metabolic reprogramming of tumor cells (78,79). mTOR is

also an upstream activator of HIF-1 and c-Myc in tumor cells, and high levels of Akt and mTOR activity lead to high HIF-1 activity and adaption to hypoxia (78,79).

Adaption to hypoxia in glycolysis reprogramming in tumors. Hypoxia is a common feature of various malignant tumor cells (59), and the adaption of tumor cells to hypoxia is predominantly mediated by HIF-1, a key transcription factor that regulates nine of 10 enzymes involved in glycolytic energy metabolism (80). In addition, an accumulation of pyruvate and lactate derived from high rates of aerobic glycolysis may promote hypoxia-inducible gene expression independently of hypoxia via stimulating the induction and stability of HIF-1 α (81). Similarly, p53, which is mostly known for its tumor suppressor properties, is implicated in the metabolic adaption of tumors through decelerating glycolysis and accelerating mitochondrial oxidative respiration (82,83). As a ubiquitin ligase, mouse double minute 2 homolog (Mdm2) may be phosphorylated by Akt and mediates the ubiquitylation and proteasome-dependent degradation of p53 (84).

Summary of molecular mechanisms mediated by glycolytic metabolism in tumors. In conclusion, relevant transporters and receptors on tumor cells integrate signals from growth factors, cytokines and nutrient availability in the tumor microenvironment to activate the PI3K/Akt/mTOR signaling pathway, which regulates the expression of various transcription factors, including c-Myc, HIF-1 and p53, leading to the reprogramming of glucose metabolism in tumors. Of note, a reciprocal interaction exists between molecular signaling pathways regulating c-Myc, HIF-1 and p53, forming a complicated and intricate regulatory network controlling the metabolic switch in tumors (85).

4. Glucose metabolic competition between tumor cells and tumor-infiltrating immune cells and consequent immune escape

A number of mechanisms for the immune evasion of tumor cells have been elaborated (86), including the downregulation of tumor-associated antigen and costimulatory molecule expression, and the upregulation of inhibitory immunomodulatory molecules and immunosuppressive cells (3,9,87). The metabolic interplay between tumor cells and infiltrating lymphocytes has been suggested to be an important metabolic mechanism underlying immunological escape of tumor cells (88) (Fig. 2). The similarity of tumor cell and activated lymphocyte metabolism is not coincidental, as it is essential that their metabolism matches the functional demands of the cells. Rapid growth and proliferation are necessary for tumor cells and activated lymphocytes; therefore, they preferentially select the more biosynthesis-efficient aerobic glycolysis and anabolism over the energy-oriented mitochondrial OXPHOS.

Role of tumor-imposed glucose restriction in antitumor immune escape. It is likely that intense nutrient competition exists between tumor cells and TILs in the tumor microenvironment, as tumor cells may deplete nutrients due to their dependence on enhanced aerobic glycolysis (10). This tumor-imposed glucose restriction may lead to TIL

dysfunction due to reduced glucose uptake and metabolic reprogramming. In an established mouse model of progressing and regressing tumors, the progressing tumors exhibited higher rates of glycolytic activity compared with regressing tumors, suggesting that progressing tumors consume more glucose. Consistently, T cells in progressing tumors exhibited decreased phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and S6 kinase compared with that in regressing tumors. However, Tregs and M2 macrophages, neither of which depend on enhanced aerobic glycolysis, but rather on fatty acid oxidation, are unaffected by the glucose-depleted tumor microenvironment, and may suppress the antitumor immune response.

Role of metabolites produced by enhanced tumor glycolysis in tumor-infiltrating immune cell dysfunction. In addition to tumor-imposed nutrient limitation for tumor-infiltrating immune cells, metabolites, including the enhanced lactate production by tumors due to increased dependence on glycolysis (59,62,89), are suggested to be key metabolic components in the communication between tumor cells and tumor-infiltrating immune cells (34,90). It is important that the excess cellular lactate produced by tumors is exported, mainly by MCTs (MCT1/MCT4), in order to prevent acidosis in tumor cells, which leads to the accumulation of lactate in the tumor milieu (91). Lactate has been shown to promote cancer cell stemness (92) and metastasis (93) through the increased production of several tumor progression-promoting factors, including transforming growth factor (TGF)- β (94,95), hyaluronic acid and CD44 (96). In addition to its direct effect on tumor cells, lactate act as an immunomodulator, mediating the immune evasion of tumor cells (97). The exogenous lactate treatment of natural killer (NK) cells inhibited their cytotoxicity directly and indirectly by increasing the number of myeloid-derived suppressor cells (MDSCs), which may repress NK cell function (12,98). By contrast, LDHA-deleted pancreatic cancer cell xenografts, with a defect in lactate production, exhibited improved cytolytic function of NK cells in C57BL/6 mice, with higher expression of perforin and granzyme and a decreased number of MDSCs in the spleen (12,98). Furthermore, the immunomodulatory effects of lactic acid have been demonstrated not only for dendritic cells, but also for T cells (13). However, the export of excess lactic acid by activated T cells is inhibited due to the lactic acid gradient between the cytoplasm and extracellular milieu, due to the accumulation of lactic acid secreted by surrounding tumor cells with high rates of glycolysis. To conclude, the accumulation of tumor-derived lactate in the extracellular milieu may lead to a metabolic obstruction in cytotoxic T lymphocytes (CTLs); subsequently, CTLs become hyporesponsive, exhibiting decreased proliferation, cytokine secretion and cytotoxic activity (13,99,100). Tregs and M2 macrophages are not affected by the presence of high levels of lactate, as their distinct metabolic program relies mainly on fatty acid oxidation rather than aerobic glycolysis (48).

An acidic tumor microenvironment resulting from upregulated tumor glycolytic metabolism leads to decreased antitumor immunity. As MCT1 and MCT4 are H⁺/lactate symporters, lactate efflux via MCT1/MCT4 is accompanied by H⁺ transport, which eventually creates an acidic microenvironment (97).

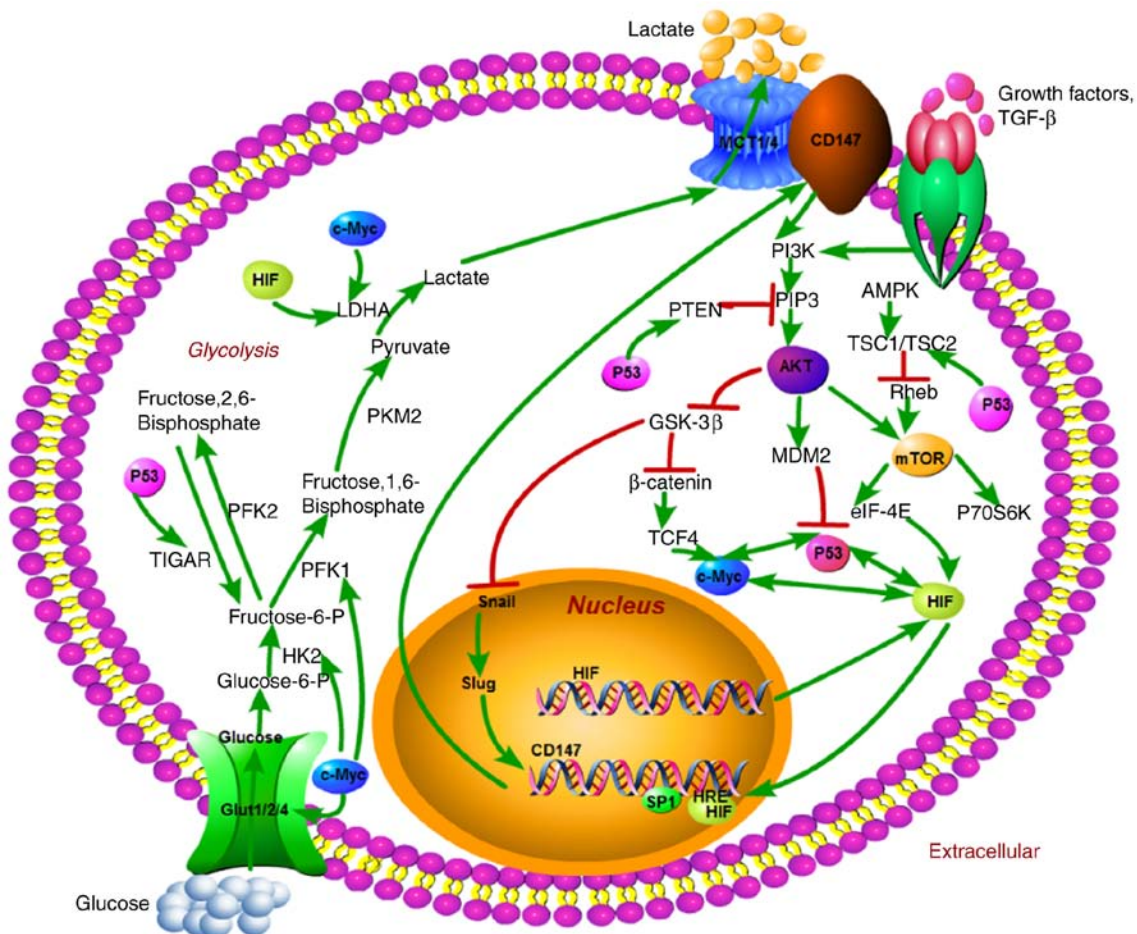


Figure 1. Schematic representation of the regulation of altered glucose metabolism associated with the upregulation of CD147 and the underlying molecular mechanism in tumors. Green arrows represent stimulation/activation and red ends represent inhibition. CD147, cluster of differentiation 147.

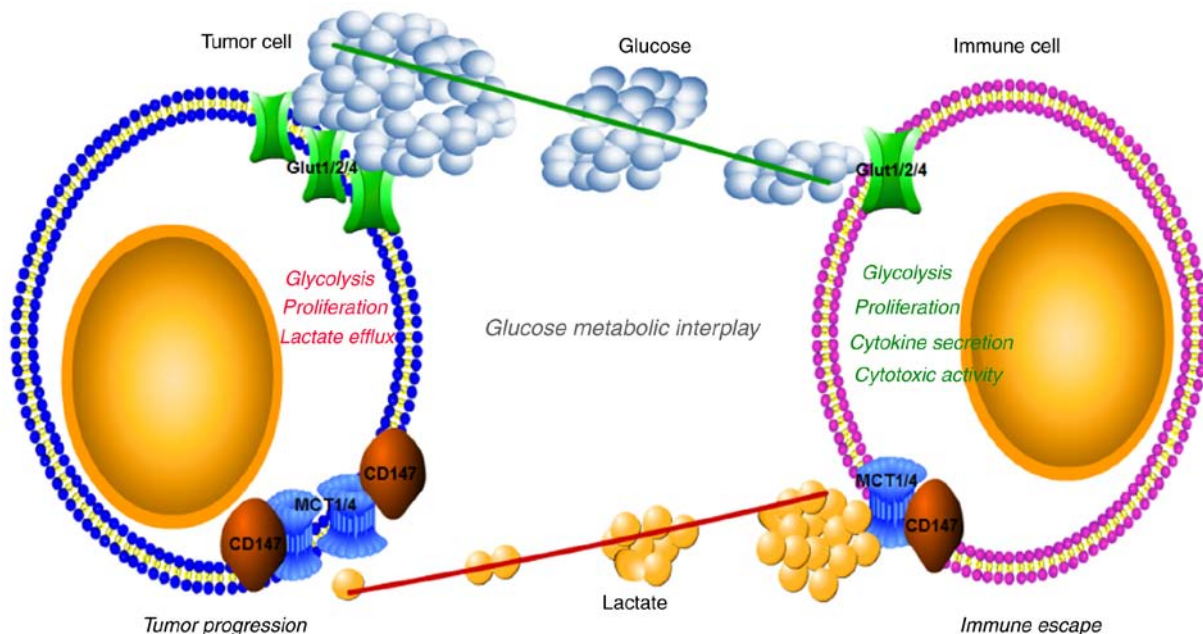


Figure 2. Schematic representation of immune evasion mediated by glucose metabolic interplay between tumor cells and immune cells associated with overexpression of CD147 on tumor cells. Tumor-imposed glucose restriction mediated by enhanced aerobic glycolysis in tumor induced by the overexpression of CD147 may lead to reduced glucose uptake by tumor-infiltrating immune cells and subsequent immune cell dysfunction. In addition, the increased lactate production by tumor cells due to enhanced aerobic glycolysis may be a key metabolic factor in the communication between tumor cells and infiltrating immune cells through inhibiting glycolysis, proliferation, cytokine secretion and cytotoxic activity of immune cells. The balance of glucose metabolic interplay is shifted towards tumor cells, and the upregulated expression of CD147 in tumor cells significantly contributes to tumor progression and immune evasion. Red, upregulation; green, downregulation. CD147, cluster of differentiation 147; MCT1/4, monocarboxylate transporter 1/4; GLUT1/2/4, glucose transporter 1/2/4.

Extracellular acidosis is characteristic of the tumor microenvironment, and the local acidification allows tumor cells to be aggressive via increased extracellular matrix degradation and enhanced survival and metastasis (101,102). In addition to the promoting effect of extracellular acidosis on tumor progression, the acidic microenvironment is important in the immune evasion of tumor cells mediated by immune cell dysfunction. Interferon (IFN)- γ , as a critical mediator of the differentiation of Th1 and Th2 cells, promotes Th1 polarization and inhibits Th2 differentiation (103). However, IFN- γ is acid-labile and likely to be denatured in an acidic milieu, which diverts Th-cell differentiation from the antitumor Th1 lymphocytes towards the tumor-promoting Th2 lymphocytes (104). HIF-1, a key transcription factor predominantly mediating the adaptation of tumor cells to hypoxia, may not be degraded under sufficient oxygen supply (105), thereby contributing to HIF-dependent glycolytic reprogramming in tumor cells and, consequently, decreased antitumor immunity.

HIF-1-associated signaling pathway in immune cells directly contributes to tumor immunosuppression. Given the profound effect of HIF-1 on gene regulation, T-cell differentiation is likely controlled by HIF-1 (15,22). Furthermore, TGF- β 1 may stabilize HIF-1 through the inhibition of prolyl hydroxylase 2 under hypoxic conditions (106). A screening of key transcription factors for T-cell differentiation during inflammatory hypoxia of the mucosa revealed forkhead box (FOX)P3 as a direct target of HIF-1, and it has also been demonstrated that the hypoxic induction of FOXP3 and accumulation of Treg require both HIF-1 and TGF- β 1 (107). HIF-1 has also been identified as a decisive factor in T-cell differentiation to Th17 or Tregs by promoting Th17-polarization and inhibiting Treg differentiation (22,51). TGF- β 1 may be induced in hypoxia (108), and is also key role the differentiation of Th17 and Tregs (109). It is reasonable to hypothesize that the Th17/Treg balance is an integral outcome of HIF-1, TGF- β 1 and inflammatory cytokine interplay in the local tumor milieu (22,51,107). The exposure of human breast and prostate cells, and mouse melanoma and mammary carcinoma cells to hypoxia resulted in the upregulation of programmed cell death ligand-1 (PD-L1), an important immunoinhibitory molecule, on the surface of tumor cells in an HIF-1-dependent manner, which eventually contributed to tumor cell evasion from antitumor immunity via the increased apoptosis of CTLs due to the enhanced PD-1/PD-L1 interaction (110). Although the mechanisms underlying increased the expression of PD-L1 on tumor cells remain to be fully elucidated, the hypoxia-induced upregulation of PD-L1 on tumor cells may represent a novel mechanism responsible for hypoxia-mediated immune evasion of tumor cells. In addition to the previously known inhibitory effects of TGF- β 1 on T-cell differentiation and function, TGF- β 1 has also been found to mediate T-cell hyporesponsiveness, in part through the enhanced expression of PD-1 on tumor-infiltrating antigen-specific T cells induced by mothers against decapentaplegic homolog 3 (Smad3)-dependent signaling (111,112).

5. Overexpression of CD147 in tumors and regulatory signaling pathways

CD147 may significantly contribute to tumor growth, invasion, metastasis and angiogenesis (29,30,113), particularly

in human hepatocellular carcinoma (HCC), mainly via triggering the production of MMP and interacting with various ligands involved in neoplastic cell behavior, including integrin α 3 β 1 (114) and α 6 β 1 (115), and Annexin II (116). The non-metabolic molecular mechanisms responsible for the tumor progression associated with the upregulation of CD147 were discussed in a previous review (38).

Metabolic mechanisms responsible for tumor progression associated with the upregulation of CD147. In addition to non-metabolic molecular mechanisms responsible for the tumor progression associated with the upregulation of CD147, a metabolic molecular basis has become a focus of investigations. Blocking CD147 with a targeted monoclonal antibody or silencing CD147 by small interfering RNA has been shown to result in a marked decrease in glycolytic energy metabolism (117,118). Consistently, a study by Huang *et al* demonstrated that CD147 acts as an important regulator of cell proliferation through promoting glucose metabolic reprogramming by the post-transcriptional inhibition of p53 via the activation of PI3K/Akt/Mdm2 signaling promoted by MCT1-induced lactate export in HCC (119) (Fig. 1). CD147 is increasingly recognized as being implicated in glucose metabolism reprogramming in tumors through gain/loss-of-function studies (92,120).

Regulatory signaling pathway for the expression of CD147. According to the regulation of CD147, it is well established that the tumor microenvironment serves an important role in the overexpression of CD147 on the surface of tumor cells. As is known, TGF- β 1 induces epithelial-to-mesenchymal transition (EMT) via Smad-dependent and -independent signaling pathways (121-123). A study by Wu *et al* demonstrated a positive correlation between the expression of CD147 and typical EMT markers, revealed that CD147 is a Slug target gene, and demonstrated that the upregulation of CD147 involves activation of the PI3K/Akt-GSK3 β -Snail-Slug signaling pathway through the stimulation of TGF- β 1 (124). A series of transcription factors have been found to be implicated in the fundamental metabolic adaptation of tumors to hypoxia, among which HIF-1 is critical to this process (125,126). HIF-1 is a heterodimer that consists of a constitutively expressed HIF-1 β subunit and an oxygen-sensitive HIF-1 α subunit (127). Under hypoxic conditions, HIF-1 α binds to a conserved DNA consensus, referred to as hypoxia-responsive element (HRE), on the promoters of numerous hypoxia-responsive genes. There are two HIF-1-binding sites and three specificity protein 1 (SP1)-binding sites in the 3' and 5' flanking regions of the CD147 gene, respectively (128,129). Consistently, a genome-wide chromatin immunoprecipitation-on-chip assay and immunohistochemical staining identified CD147 as a novel hypoxia-responsive molecule. The identification of key molecules engaged in epithelial solid tumor glycolytic switch confirmed that the upregulation of CD147 was mainly mediated through the combined effect of HIF-1 α and SP1 on activation of the CD147 promoter (120). A study by Kong *et al* also reported that upregulation of the expression of CD147 was mediated by promoter hypomethylation through increased SP1 binding in human HCC and lung cancer (127,130).

In conclusion, the signaling pathways responsible for the overexpression of CD147 on tumor cells mainly include TGF- β 1 and HIF-1 α , which are pivotal in tumor glycolysis and immunosuppression.

6. Therapeutic potential and clinical implications of metabolic intervention in tumors

Successful chemotherapeutic tumor treatment generally depends on the rapid proliferation of tumor cells. However, undesirable side-effects on normal proliferating cells are inevitable due to the non-specific nature of this treatment. Therefore, therapeutic strategies based on specifically targeting the 'metabolic transformation' of tumor cells may be a preferred approach (63). Various potential agents targeted against the altered metabolism of tumor cells are currently in clinical trials, and several more are under development.

Therapeutic drugs for manipulating the metabolic activity of immune cells to prevent immune cell hyporesponsiveness in tumors. Apart from tumor cells themselves, manipulating the metabolic activity of immune cells to prevent immune cell hyporesponsiveness in tumors is currently considered a promising approach in cancer therapy (11,19,20). The fundamental principle of modulating the metabolism of T cells is to favor anabolic glycolysis rather than catabolic oxidative respiration. Specific antibodies against nutrient transporters have been confirmed as potential pharmacological agents targeting T-cell metabolism. For example, blockade of GLUT1 on T cells has been found to decrease glucose uptake, thus leading to T-cell dysfunction (17). The blocking of co-inhibitory receptors has been suggested as a promising immunotherapy option for enhancing antitumor immunity to eliminate tumor cells (131-134). Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-1, two well-known inhibitory receptors on T cells, are induced upon T-cell activation to control and moderate excessive immune responses, acting as checkpoints (131). However, CTLA-4 and PD-1 signaling have been shown to restrict T-cell activation and function by downregulating aerobic glycolysis (10,135). Therefore, it is hypothesized that checkpoint blocking may relieve the suppression of antitumor immunity, in part through remodeling T-cell metabolic programming to enhance nutrient uptake and glycolytic metabolism, consequently restoring their capacity to kill tumor cells (11). PD-L1 also has a PD-L1 and PD-1 interaction-independent metabolic function (111,136). PD-L1 on tumor cells is important for Akt/mTOR signaling, which in turn increases the rate of glycolysis through promoting the translation of glycolytic enzymes. Blocking PD-L1 may directly decrease glycolysis in tumors, increasing the nutrient availability in the extracellular tumor milieu for infiltrating lymphocytes (10,11).

Direct and indirect therapeutic drugs targeting metabolic adaptation of tumor cells. In general, therapeutic drugs targeting the metabolic adaptation of tumor cells may be divided in two categories, namely direct and indirect. Indirect drugs target aberrant signaling pathways relevant to metabolic transformation in tumor cells, including the HIF-1 α (137), c-Myc (67,138), PI3K/Akt/mTOR (139-141) and AMPK (142)

pathways. For example, metformin, a drug originally designed to treat patients with type 2 diabetes (143), may activate the AMPK signaling pathway to oppose mTORC1, subsequently decreasing glycolytic metabolism and increasing OXPHOS in tumor cells to control tumor progression (144,145). Consistently, patients with type 2 diabetes who were treated with metformin were more likely to remain cancer-free over 8 years compared with those who received other treatments (146,147). Metformin is currently in phase I and II clinical trials for cancer therapy. Direct drugs comprise antagonists against multiple metabolic enzymes and several metabolites in glucose, amino acid, lipid and nucleotide metabolism (63). This review focuses on glycolytic metabolism. Almost all enzymes involved in every stage of glycolysis may represent potential targets, particularly tumor-specific enzyme isoforms and glycolytic metabolites, including PKM2 (129,148) and lactic acid (90,102). In terms of CD147, it has been reported in patients with HCC that targeted radioimmunotherapy with ¹³¹I-labeled HAB18 F(ab')₂ metuximab monoclonal antibody injection (licartin), which is a radiolabelled anti-CD147 monoclonal antibody, effectively prevented the recurrence and metastasis of HCC following hepatectomy and liver transplantation (149). Based on the evidence described above, it is reasonable, to a certain extent at least, to attribute the antineoplastic capacity of licartin to its ability to inhibit the glycolytic metabolism of HCC cells. Combination therapy of ¹³¹I-labeled metuximab and other metabolic transformation-targeting drugs may be more beneficial for antitumor treatment compared with monotherapy.

Targeted delivery of therapeutic drugs and combination treatment with metabolic intervention and antitumor immunotherapy. Regardless of the modulation of cellular metabolism in tumor cells or T cells, targeted delivery of specific drugs in the body is crucial for preventing off-target effects. Transporter-facilitated drug uptake (150,151), bi-specific antibodies (152,153) and nanoparticle-mediated delivery (154,155) have been developed to optimize drug efficacy. Optimal Teff function in the tumor microenvironment is necessary for successful adoptive T-cell immunotherapy. Combination treatment comprising metabolic intervention and adoptive T-cell immunotherapy appears promising for metabolic reprogramming of T cells to exert effective antitumor immunity (19,20). As reported previously, CD147 serves an important role in the reprogramming of glucose metabolism and cell proliferation in HCC cells (119), and a targeted radio-labeled anti-CD147 monoclonal antibody (licartin) effectively prevented the recurrence and metastasis of HCC following hepatectomy and liver transplantation (149). The blocking of CD147 inhibited the enhanced glycolysis of HCC cells and contribute to improved antitumor immunity in the tumor microenvironment, which is exactly what current endeavors are aiming to prove. Combination therapy comprising CD147 intervention and tumor immunotherapy is likely to lead to more marked antitumor effects than monotherapy. However, the timing of adoptive Teffs entering the local tumor milieu is an important issue requiring consideration. Using inhibitors of glycolysis prior to the adoptive transfer of T cells may assist in remodeling metabolic function in T cells in a hospitable tumor milieu with nutrient repletion (19,20).

7. Conclusions

CD147 exhibits high expression on the surface of a variety of malignant tumor cells, and serves an important role in neoplastic cell behavior via non-metabolic and metabolic molecular mechanisms. Specifically, the involvement of CD147 in tumor glucose metabolism reprogramming has been suggested, as CD147 can assist in the folding, stability, membrane expression and functionality of MCTs as a chaperone in the transport of monocarboxylates, including L-lactate, across the plasma membrane in tumor glycolysis. The metabolic interplay between tumor cells and infiltrating lymphocytes has been increasingly recognized as an important metabolic mechanism underlying the immune escape of tumor cells, including intense competition for nutrients between tumor cells and TILs in the tumor microenvironment, and an accumulation of tumor-derived lactate in the extracellular milieu. HIF-1 α , c-Myc, PI3K/Akt/mTOR and AMPK signaling are considered to be important metabolic pathways responsible for metabolic reprogramming and antitumor immunoediting in tumors. Therefore, the manipulation of cellular metabolism may be of value for the treatment of immunological disorders and tumor immunotherapy.

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Availability of data and materials

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Authors' contributions

XL and WX conceived, designed the study and wrote the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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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