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Title: Linking Tumor Glycolysis and Immune Evasion in Cancer: Emerging Concepts and Therapeutic Opportunities

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Abstract

Metabolic reprogramming and immune evasion are two hallmarks of cancer. Metabolic reprogramming is exemplified by cancer's propensity to utilize glucose at an exponential rate which in turn is linked with "aerobic glycolysis", popularly known as the "Warburg effect". Tumor glycolysis is pivotal for the efficient management of bioenergetics and uninterrupted cancer growth. Mounting evidence suggests that tumor glycolysis also plays a key role in instigating immunosuppressive networks that are critical for cancer cells to escape immune surveillance ("immune evasion"). Recent data show that induction of cellular stress or metabolic dysregulation sensitize cancer cells to antitumor immune cells implying that metabolic reprogramming and immune evasion harmonize during cancer progression. However, the molecular link between these two hallmarks of cancer remains obscure. In this review the molecular intricacies of tumor glycolysis that facilitate immune evasion has been discussed in the light of recent research to explore immunotherapeutic potential of targeting cancer metabolism.

1. Introduction

The ability of cancer to switch to different energy-producing pathways (i.e., metabolic reprogramming) and its capacity to escape immune surveillance (i.e., immune evasion) have recently been recognized as molecular signatures of cancer [1]. These two cancer-related phenomena draw parallelism not only in their designation as molecular signatures of tumor progression but also in their historical background (Fig. 1). The tendency of cancer cells to utilize glucose via the process of glycolysis irrespective of oxygen availability is known as aerobic fermentation or aerobic glycolysis. This aerobic glycolysis, later referred as “tumor glycolysis”, has been known since the 1920s [2]. More importantly, the notion that cancer cells are sensitive to metabolic interference (e.g. antimetabolites) has also been known for more than half a century [3--5]. Analogously, the earliest indication that stimulating the immune system could affect cancer cells dates back to the 19th century, when Dr. Coley reported that injection of bacterial cultures into patients with inoperable sarcoma markedly reduced tumor burden [6,7]. Despite early progress, research on these two key observations of cancer (i.e.) “tumor glycolysis” and “immune evasion” was eclipsed by several factors, including the global impact of the World Wars and the emergence of the genomic era. Incidentally, both of these hallmarks have witnessed a revival of research interest in the past couple of decades, and the development of potential therapeutic strategies to target these two hallmarks have gained momentum (Fig.1).

Though metabolic reprogramming and evasion of immune surveillance are distinct processes, emerging reports indicate that they may be fundamentally linked [8--10]. Thus, there is a renewed interest to exploit the link between these two cancer-related processes in order to develop potent anticancer therapeutics. This review discusses, in light of recent research, the molecular intricacies of tumor glycolysis that facilitate immune evasion, and expands on the clinical relevance and therapeutic potential

of targeting energy metabolism to augment immunotherapy. From an immunological perspective, natural killer (NK) cells are discussed in greater detail primarily due to the accumulation of data that links energy metabolism with cancer's sensitivity to NK cells [11--13]. In conclusion, this review deliberates current challenges, potential opportunities and future directions of research that may be critical to significantly improve the outcome of immunotherapy for cancer.

2. Tumor Glycolysis

In general, metabolic reprogramming refers to the rewiring of metabolic circuitry. Several elegant reviews have underscored the role and significance of altered energy metabolism in cancer progression [14--16], whereas this review will focus only on recent advancements in understanding the immunomodulatory aspects of glucose metabolism in cancer. The ability to consume and oxidize glucose at an accelerated rate is a biochemical phenotype of majority of malignancies. In fact, this phenomenon is so prominent and frequent that it is currently exploited in the clinical diagnosis of cancer through the positron emission tomography (PET) [17].

Biochemically, oxidation of glucose via mitochondria (oxidative phosphorylation; OxPhos) yields 18 times more energy (adenosine triphosphate; ATP) molecules than glycolysis. However, it is increasingly evident that aerobic-glycolysis, though mathematically disadvantageous in terms of net ATP production, is preferred by cancer cells to achieve other biological requirements of proliferation [18]. For example, glycolysis provides the basis for several biomacromolecules (e.g. ribose 5 phosphate, NADPH) that participate in anabolic processes and serve as building blocks [19]. Next, although the net energy outcome of glycolysis is lesser than OxPhos-mediated hydrolysis of glucose molecule, strikingly, the rate of glycolysis is 100 times faster than OxPhos [20]. In other words, in a defined period, a glycolytically

active cancer cell consumes more glucose molecules than a corresponding non-glycolytic, OxPhos cancer cell. Physiologically this provides a competitive advantage to exponentially-expanding cancer cells over the surrounding slow-dividing and/or OxPhos-dependent cells [8,18].

Furthermore, experimental evidence also reveals that such glycolytic cancer cells provide additional advantages to cancer growth by selective conditioning of the tumor microenvironment (TME). For instance, the extracellular accumulation of lactate derived from glycolysis alters the TME by generating acidic pH which is detrimental to normal cells. Note, such acidic TME renders resistance to therapy and remains a major impediment for successful cancer treatment [21]. Arguably, excessive accumulation of lactate may also be harmful to cancer cells. Intriguingly, the existence of a metabolic symbiosis between glycolytic cancer cells and mitochondrially active, OxPhos cancer cells have been reported. The metabolic symbiosis wherein glycolytic cells release lactate while the non-glycolytic (OxPhos) cancer cells import the lactate and use via TCA cycle has been ascribed as an adaptive mechanism to support tumor cells with diverse metabolic phenotypes [22]. Thus, metabolic reprogramming fulfils cancer's energy/nutrient requirements, enabling successful competition with non-cancerous cells, and facilitating the development of the critical barrier, the TME.

Furthermore, a growing body of literature suggests that cancer-associated fibroblasts (CAFs), which are in the vicinity of TME also play a critical role in supporting cancer growth. Cancer cells have been known to modulate the metabolic phenotype of CAFs from OxPhos to glycolysis. This metabolic switch in CAFs has been shown to be achieved by the release of cancer-derived reactive oxygen species (ROS) [23] or signaling molecules (e.g. TGF-beta) [24] in to the TME which in turn generates oxidative stress in CAFs necessitating them to adopt to glycolysis. Induction of such metabolic reprogramming in CAFs

has been found to be advantageous to cancer cells that rely on OxPhos. In brief, the lactate produced by such glycolytically active CAFs are imported by mitochondrially-active cancer cells for further oxidation via OxPhos. This phenomenon, referred as “reverse Warburg effect”, underscores a strong link between cancer and its associated fibroblasts (CAFs) (Fig 2a) [25]. Nevertheless, in glycolytic tumors, it is also plausible that CAFs may import lactate from the TME and utilize it via OxPhos [in the absence of oxidative stress], thus preventing the accumulation of toxic levels of lactate. More importantly, utilization of cancer-derived lactate by CAFs to meet their energy requirements may also mitigate their competition for glucose consumption resulting in the accumulation/availability of glucose for cancer cells (Fig 2a). Similar metabolic competition has already been implied between cancer cells and immune cells (e.g. T cells), and also between normoxic and hypoxic cancer cells [22,26]. Thus, CAFs which are an integral component of TME facilitate cancer growth.

3. Immune evasion

It is increasingly evident that cancer cells implement immunosuppressive networks to escape immune surveillance and challenge potential antitumor immune cells [27]. In general, such immunosuppressive mechanisms involve either immune system-related suppressors (e.g. myeloid-derived suppressive dendritic cells (MDSCs) and macrophages (tumor associated macrophages, TAMs)) or cancer-associated factors [27] (Fig 2b). Cancer-associated immune suppressive mechanisms include a myriad of factors. First, cancer cells emulate expression of immune check-point molecules or inhibitors that are displayed by normal healthy cells to prevent autoimmunity (an aberrant immune response that leads to self-destruction of healthy cells). For example, cytotoxic T lymphocytes associated antigen 4 (CTLA4) and programmed cell death protein (PD-1) are some of the known immune check-point regulators that are activated by cancer [28] to evade immune recognition.

Next, cancer cells down-regulate the expression of surface antigens to escape immune-surveillance. A typical prototype is the deregulation of major histocompatibility complex (MHC) class antigens. Prominent among them is the MHC class I chain (MIC)-related protein A or B (MIC-A/B), the natural ligands for the receptor, NKG2D on NK cells, which enable NK cells to recognize diseased or abnormal cells for elimination. Paradoxically, cancer cells alleviate the surface expression of MIC-A/B [29]. Thus down-regulation of surface antigens by shedding or cleavage enables cancer cells to escape detection and elimination by NK cells. Finally, as discussed elsewhere in the review, cancer-dependent alteration in the TME acts as a barrier to any infiltrating antitumor immune cells [30].

4. Link between tumor glycolysis and immune evasion

Though lactate produced by glycolytic cancer cells has been implicated in the suppression of anticancer immune cells, the mechanism involved in such inhibition remained unclear. The lack of understanding was primarily due to the fact that immune cells (e.g. T lymphocytes, macrophages) also switch to glycolysis and produce lactate during their growth or maturation/activation [31,32]. Thus, lactate-exposure is not completely foreign to immune cells, and *per se* may not be cytotoxic to immune cells. Nevertheless, recent reports indicate several possible mechanisms that facilitate lactate-mediated inhibition of antitumor immune functions. First, the prevalence of excessive amounts of lactate in the TME abrogates the release/export of lactate by glycolytic-T cells by a gradient-dependent inhibitory mechanism (Note: T cells switch to glycolysis during growth/activation, hence it is critical to prevent intracellular accumulation of lactate). Thus, the high concentration of lactate in TME affects the rate of proliferation and function of T cells by disturbing their intracellular pH due to the accumulation of lactate [31]. A similar mechanism has been shown to modify dendritic cells (DCs) affecting their maturation [33].

Next, lactate uptake by macrophages results in the induction of the gene, ARG1 (arginase-1) [34] which in turn has been known to inhibit T cell activation and proliferation [35]. Besides the lactate acidosis, glycolytic cancer cells also compete with proliferating T cells for increased glucose uptake, as T-cells also rely on glycolysis for their maturation and immunological functions. Other immune cells such as NK cells and MDSCs have also been affected by lactate resulting in a marked alteration in their efficiency [13,36]. Thus, by reprogramming energy metabolism to a glycolytic phenotype, cancer cells effectively interfere with antitumorigenic functions of immune cells, and achieve immune evasion.

Besides the evasion of immune surveillance, cancer cells also influence the physiology of selective immune cells to facilitate uninterrupted cancer growth. In other words, a functional and/or biochemical link exists between cancer cells and immune cells wherein the former impacts the later in its favor. For example, among T cells, T_H1 subpopulation express IFN- γ and enhance the antitumor activity of “M1” macrophages [37]. On the contrary, the T_H2 subpopulation secrete interleukins (e.g. IL-4) and activate B cells with tumor promoting inflammatory response [38]. Interestingly, IFN- γ inhibits the differentiation of T_H2 cells leading to the suppression of pro-inflammatory, pro-tumorigenic actions of T_H2. Paradoxically, IFN- γ is sensitive to low-pH and acidic conditions [39]. Hence, the acid-labile IFN- γ is unstable and dysfunctional in acidic, low-pH TME. Consequently, the T cell maturation process results in a divergence of T-helper cells into T_H2 cells that are pro-tumorigenic and prevents the maturation of antitumor, M1 macrophages [10]. Thus acidic microenvironment plays an immune suppressive role. Next, it is well established that the stabilization of hypoxia inducible factor (HIF)-1 α , a key regulator of tumor glycolysis [40], facilitates the up-regulation of myriad of genes that regulate cancer-related processes such as angiogenesis [41]. Biologically, HIF-1 α stability requires hypoxic condition, and under

normoxia it is down-regulated by VHL-dependent regulatory mechanism [40]. Interestingly, it has come to light that even in the presence of sufficient oxygen (normoxia) an acidic environment could still facilitate the stabilization of HIF-1 α , and activate its target oncogenes [42]. Thus an acidic TME maintains the stability of HIF-1 α facilitating the expression of VEGF which in turn could expand immunosuppressive MDSCs [43] to promote uninterrupted tumor growth.

It is known that cancer-derived products (e.g. lactate, H⁺) impact TME. Nevertheless, TME may also influence cancer's metabolic activity in a manner that favors tumor growth [8--10]. Besides, TME also acts as a critical barrier between the tumor and any non-cancer tissues, or anticancer agents (e.g. chemotherapeutics, immune cells). Many elegant reviews have delineated the role and significance of TME in cancer progression and therapeutic-resistance. From an immune-modulator perspective, TME has been shown to be one of the challenges that affects the efficacy of tumor-specific antigen presenting T cells. Cancer-specific T cells have been known to be primed to express inhibitory receptors that favor or protect cancer cells [21]. This has been further supported by the fact that such inhibited or suppressed tumor infiltrating T cells regain their anticancer efficacy in the absence of TME such as *in vitro* culture condition [44]. Data also show that lactic acid in the TME suppresses T-cell function [45] and also functionally polarizes tumor-associated macrophages [46]. Collectively, existing data convincingly demonstrate that metabolic reprogramming and immune evasion harmonize to drive cancer progression (Fig 3).

A growing body of evidence demonstrate that the glycolytic phenotype of cancer cells suppress cancer-targeting immune cells [10]. Although the product of tumor glycolysis, i.e. lactate contributes to the acidity and low- pH of TME, other factors such as protons [H⁺], carbonic acids among others also play

inevitable role in the maintenance of TME. From an immunotherapy perspective, such low-pH TME has been shown to suppress the physiology of cytotoxic and antigen-presenting cells [47]. Hence targeting TME has been contemplated as one of the effective approaches to improve the efficacy of anticancer therapeutics [48]. Notably, emerging data underscore the significance of neutralization of TME and its impact on therapeutic outcome. Systemic buffering with bicarbonate has been shown to improve the pH of TME [49,50]. More importantly, neutralization TME pH by systemic buffering enhanced outcome of anticancer immunotherapy [51]. Recently, such neutralization efforts to reverse tumor acidosis have shown improved outcome in NK cell mediated anticancer effects as well [52]. Together these data indicate that targeting the low-pH of TME by buffering provides a window of opportunity to achieve better outcome.

Among the major determinants that govern TME, accumulating data underscore the significance of proton exchangers/ transporters, monocarboxylate transporters (MCTs) and carbonic anhydrases (CAs) among others [53]. Particularly, targeting the proton exchanger/transporters have gained attention as the outcome enabled intracellular acidification of cancer cells leading to cytotoxicity. Correspondingly, disruption of low-pH of TME has been endeavored by the inhibition of proton pumps of cancer cells which play critical role in the excretion or extracellular accumulation of protons. Recent research has shown that inhibition of extrusion of acidic molecules or protons by proton pump inhibitors (PPI) affect tumor growth. Immunotherapeutically, PPIs such as esomeprazole [54] and pantoprazole [55] have demonstrated marked enhancement in the anticancer efficacy of T cells and macrophages, respectively. Thus, alteration of TME to reduce the acidity and increase the pH by either inhibition of lactate production or systemic buffering or by blockade of proton pumps may improve outcome of cancer immunotherapy.

5. Potential opportunities and future directions

An effective cancer therapeutic approach is likely to integrate targeting more than one hallmark of cancer, a “two-hit” approach. For example, if metabolic reprogramming and immune evasion are targeted by a single interventional strategy, besides sensitization of cancer cells to immune recognition; it could also delay the growth and progression due to the deregulation of cellular bioenergetics. Such “two-hit” approaches have been contemplated for targeting one of the signaling molecules, protein kinase A (PKA) isoform which plays an important role in growth and proliferation as well as tumor-induced immune suppression [56]. Targeting energy metabolism of cancer could invoke a dichotomous effect in enhancing the efficacy of immunotherapy. From a metabolism perspective, deregulation of glycolysis could affect the TME by reducing the level of lactate accumulation, resulting in improved infiltration of functionally active immune cells that can recognize and eliminate cancer cells. Concurrently, disruption of energy metabolism also induces cellular stress which in turn up-regulates the stress-inducible antigens such as MICA/B sensitizing cancer cells to NK cell mediated cytotoxicity [11] (Fig 4).

It is now well established that TME plays a critical immunosuppressive role including inhibition of T cells and NK cells [12,57]. This is relevant for both adoptive cell transfer therapy and endogenous antitumor immune cells [58]. Experimental evidence indicates that alteration of TME, particularly the pH reverses dysfunctional tumor-infiltrating T lymphocytes into functionally effective immune cells indicating that any interference with lactate secretion/accumulation into TME could render cancer cells sensitive to immune therapy [59]. Importantly, the role of altered energy metabolism, i.e. aerobic glycolysis, in affecting antitumor immune cells has also been reported [60]. In fact, a reduction in lactate level in the TME has been indicated to improve the survival as well as recruitment of antitumor T cells [13]. Noteworthy, the inhibitory or suppressive nature of TME has been of paramount importance from a

biological perspective as well. This is primarily due to the fact that other challenges like inadequate number of active T cells may be overcome by adoptive cell transfer or T-cell based therapy, whereas the suppressive nature of TME may be altered to a desirable phenotype only by specific targeting or priming of cancer cells prior to immunotherapy. Noteworthy, thus metabolic perturbation, particularly the energy metabolism would provide an opportunity to prime or alter the TME for an effective T-cell based immune therapy.

As discussed earlier, the transcription factor, HIF-1 α has also been indicated to play a critical role in cancer's immunosuppressive networks [61]. In order to achieve efficient glycolysis cancer cells up-regulate relevant glycolytic enzymes which in turn require the stabilization of *HIF-1 α* , a master regulator of tumor glycolysis [62]. Under normoxia, *HIF-1 α* is stabilized by accumulation of pyruvate [63] that is achieved by reduced OxPhos. Furthermore, while substantial level of ROS facilitates HIF-1 α stabilization, it is also sensitive to excessive ROS. Hence, a glycolytic phenotype enables to maintain diminished ROS production by down-regulation of OxPhos [12,64]. Therefore, elevating the levels of ROS by induction of cellular/metabolic stress is likely to be counterintuitive to the function of HIF-1 α , and affect cancer cells. In the light of recent literature it is evident that ADAM-17 requires HIF-1 α for the cleavage of MIC-A/B [65]. Thus any dysregulation of HIF-1 α could affect ADAM-17 which in turn could interfere with the cleavage of MIC-A/B, eventually leading to the surface retention of MIC-A/B. Experimental evidences show that lactate produced by metabolic reprogramming, and LDH enzyme secreted into the serum by cancer cells enable them to escape NK cell cytotoxicity by specific mechanisms [13,66]. Thus, future studies to target HIF-1 α , glycolytic phenotype could improve the sensitivity of cancer cells to NK cells mediated therapy.

Next, as part of the innate immune system, NK cells play a pivotal role in antitumor immune response and often considered as the first line of defense against cancer [67]. Reports indicate that NK cell mediated therapy has marked antitumor effects [68,69]. Both, autologous (host-mediated) and allogenic (adoptive cell transfer) NK cells have been investigated for their antitumor efficacy [67]. Despite promising outcomes in hematological malignancies, the success of NK cell immunotherapy in solid malignancies such as breast cancer remains challenged [70]. Recent evidence suggest induction of metabolic stress at sub-lethal or nontoxic dose of antimetabolites enhance the surface expression of MIC-A/B leading to the increased sensitivity to NK cell mediated cytotoxicity [11]. Indeed, data show that treatment with current chemotherapeutic, doxorubicin induces reactive oxygen species (ROS) levels leading to up-regulation of NKG2D ligands [71]. Thus, experimental evidences indicate that chemotherapy-mediated induction of cellular stress to up-regulate NKG2D ligands is feasible and has the potential to enhance sensitivity to NK cell mediated cytotoxicity. Clinical evidence shows that shedding of MIC-A and/ or MIC-B is associated with tumor progression and immune evasion [72] and targeting MIC-A/B using NK cells have been effective against cancer [73]. Further, research on NKG2D ligands such as MIC-A/B has gained momentum as experimental evidence indicated that in breast cancer cells HER2/HER3 pathways regulate the MIC-A and MIC-B expression [74]. Accumulating evidences also indicate that expression of MIC-AB and ULBP-2 results in a favorable outcome by extending the cancer-relapse free period [75]. Because NKG2D ligands are not widely expressed on healthy adult tissue, NKG2D ligands may present a useful target for immunotherapeutic approaches in cancer. Novel therapies targeting NKG2D ligands for the treatment of cancer have shown preclinical success and are poised to enter into clinical trials [76]. The emerging concept of induction of metabolic stress by a metabolic inhibitor to enhance anticancer immune response in principle emanates from the fact that MIC-A/B are stress inducible targets [77,78] and cancer cells'-dependence on altered energy metabolism is a

potential target [14]. In addition to the depletion of intracellular energy, metabolic targeting is also known to cause endoplasmic reticulum stress [79] and redox imbalance [80]. Thus any interference in energy metabolic pathway could impose physiological stress, which in turn could promote the induction of MICA/B. Recently, we reported that induction of cellular stress by targeting energy metabolism enhances NK cell cytotoxicity [11]. Although the proof-of-principle has been tested *in vitro* and *in vivo*, the translational potential and further progress of this strategy relies on robust validation in clinically relevant models.

Immunomodulatory antigens of cancer cells play an indispensable role in the evasion of antitumor immune response as well as surveillance (e.g. PD-1 ligand). Hence specific targeting of such altered antigens is contemplated to improve immune therapy [81]. Such check point inhibitors have been implicated as desirable targets to enhance effective immunotherapy [82]. Furthermore, the potential of screening such immunomodulatory factors to predict the probable responsiveness of a patient to immunotherapy has also been suggested [83]. Thus, antibody-based blockade of these immune checkpoints have shown encouraging results and such therapeutics (e.g. Ipilimumab, pembrolizumab) have also been approved by the Food and Drug Administration (FDA) for clinical use [28,58].

6. Conclusion

Metabolic reprogramming by itself is an attractive target for therapeutic intervention however the tendency of cancer cells to exhibit metabolic plasticity enables them to escape /prevent any potential anticancer effects. Experimental evidences show that glycolytic cancer cells when challenged with antiglycolytic agents such as the alkylating agent dichloroacetate or anti-LDH strategies, demonstrated survival by switching to OxPhos in the presence of molecular oxygen [84]. This indicates that the

metabolic alteration exhibited by cancer cells is a preference and not necessarily a unidirectional reprogramming. In other words, the altered metabolism of cancer cells may be rewired to OxPhos as may be necessary as long as a functionally active mitochondrion and oxygen are available. Thus, targeting metabolic phenotype alone, (e.g.) by chemotherapy, is prone to yield limited success, whereas priming cancer cells to immune therapy by metabolic deregulation rather than its abrogation to induce cytotoxicity seems to be a viable alternative. In addition, disruption of energy metabolism has been known to promote senescence in cancer cells [85] that can be used to enhance immune therapy. Noteworthy, a recent report showed that induction of senescence in tumor cells could be exploited for effective or enhanced immunotherapy by tumor-infiltrating leukocytes [86].

The novelty of metabolic stress-induced sensitization of cancer cells to NK cell mediated cytotoxicity lies in the use of sub-lethal or nontoxic, maximum tolerated therapeutic dose. This may enable us to eliminate or reduce unwanted cytotoxicity or systemic toxicity (a major impediment in the successful translation of potential therapeutics). Another major advantage of metabolic deregulation of cancer cells using sub-lethal, nontoxic dose of antimetabolite is that it would eradicate any possible deleterious side effects of conventional chemotherapeutics which have been known to affect antitumor efficiency of host immune system [87]. Noteworthy, one of the undesirable effects of potent, high dose chemotherapy is the tendency to affect immune cells or immune system which can be avoided by alternative approaches like low-dose metronomic therapy [88]. In addition, a low-dose treatment with chemotherapeutics have also been known to down-regulate the immune-suppressive mechanisms of cancer cells, a desirable outcome [89], although it is unclear if this desirable effect is due to cellular stress and/or due to the alteration of TME. Further investigations to consider therapeutics that are used for other ailments but involve

targeting altered energy metabolism and/or the TME may provide viable alternatives to enhance the outcome of immune therapy.

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Figure Legends

Fig. 1. Timeline: Some of the major milestones in the history of two hallmarks of cancer- Metabolic reprogramming (top panel) and Immune evasion (bottom panel). BCG, Bacille Calmette–Guérin; ACT, Adoptive cell transfer; FDA, Food and drug administration.

Fig. 2. An overview of the two hallmarks of cancer. (a) Left panel: Glycolytic cancer cells utilize glucose at an accelerated rate, and metabolize it through glycolytic process, (i.e.) conversion of glucose into pyruvate, followed by lactate production. The acidic lactate is excreted via the transporter, MCT4. The lactate accumulation enables cancer cells to maintain acidic microenvironment which protects against therapeutics (e.g. immune cells, chemotherapeutics). Chronic acidity is prevented by lactate uptake by normoxic (OxPhos) cancer cells or OxPhos-fibroblasts that metabolize lactate via TCA cycle for their metabolic needs. Indirectly this results in reduces the glucose utilization by other cells in the vicinity of glycolytic tumor eventually facilitating more glucose availability for glycolytic cancer cells [22]. Glycolytic phenotype enables cancer cells to metabolize glucose faster than mitochondria-dependent oxidative phosphorylation (OxPhos), also mitigates any excessive production of ROS that is associated with OxPhos. Right panel: OxPhos-competent (normoxic) cancer cells direct metabolic switch in cancer-associated fibroblasts (CAFs) by secretion of signaling molecules involved in the induction of oxidative stress. CAFs switch from OxPhos to glycolysis resulting in the production of lactate in the TME which then is utilized by OxPhos (normoxic) cancer cells via TCA cycle. This process is referred as “reverse Warburg effect” [23-24]. (b) Cancer cells escape immune recognition by multiple mechanisms; The chemokine, SDF-1 secreted by cancer cells bind with its receptor CXCR4 on tumor-associated macrophages (TAM) which inhibits cytotoxic T lymphocytes (CTL). Cancer cells also express immune-check point ligands such as PD-L1 and FasL that block CTLs function. In addition, the cytokines such as

interleukins (IL-4, 10), TGF- β and others produced by cancer cells activate myeloid derived suppressor cell and regulatory-T cell (T-reg) which control CTL's function. Finally, the surface antigen molecules such as MHC or MHC-associated molecules like MIC-A/B are down regulated or cleaved, respectively to evade immune detection by NK cells. MCT4, monocarboxylate transporter 4; ROS, reactive oxygen species; SDF-1, stromal cell derived factor-1; TGF- β , transforming growth factor- β ; PD-L1, programmed death-ligand 1; MHC, major histocompatibility complex; MIC-A/B, MHC class I chain (MIC)-related protein A or B (MIC-A/B); NK cell, natural killer cell.

Fig. 3. The link between metabolic reprogramming and immune evasion in cancer progression. (a) Functional activation of T cells requires increased glucose utilization, however, cancer cells compete with T-cells thus blocking their activity. Next, lactate produced by cancer cells maintain an acidic, low-pH tumor microenvironment (TME) which functions as an effective barrier against any infiltrating immune cells. Metabolic reprogramming to aerobic glycolysis reduces the production or accumulation of mitochondria-dependent ROS, thus preventing the up-regulation of stress-inducible MIC-A/B, the ligands of NK cells. Next, although the molecular mechanism underlying the cleavage and release of soluble MIC-A/B is poorly known, deregulation of cancer's energy metabolism has been known to elevate the expression level of MIC-A/B indicating a metabolic link. Finally, the transcription factor, HIF-1 α , a major regulator tumor glycolysis, also regulates the expression of SDF-1 which in turn affects TAM to inhibit T- cells. (b) Metabolic reprogramming or the altered energy metabolism cooperates with immune evasion during cancer progression by balancing energy requirements and evasion of immune surveillance. NK cell, natural killer cell; ROS, reactive oxygen species; MIC-A/B, MHC class I chain (MIC)-related protein A or B (MIC-A/B); HIF-1 α , hypoxia inducible factor 1 α ; TAM- tumor-associated macrophage; SDF-1, stromal cell derived factor-1.

Fig. 4. Potential metabolic targets to sensitize cancer cells to immunotherapy. Several steps of the altered metabolism can be targeted to enhance efficient infiltration of antitumor immune cells, and to sensitize cancer cells for immune surveillance. Although these metabolic pathways may be common in normal cells, the hallmark (i.e. rapid rate of glucose oxidation) exhibited by cancer cells provides a window of opportunity for increased selectivity. The objective of targeting metabolism is not to achieve complete abrogation of intermediary metabolism rather to perturb cellular bioenergetics to mitigate lactate production and/or induce metabolic stress [11--13] by sub-lethal, nontoxic dose. Thus, inhibition of LDH to reduce or prevent lactate production (indicated by red arrows/box) and/ or glycolysis (blue arrows/box) will deregulate metabolic reprogramming and necessitate cancer cells to switch to mitochondrial-dependent oxidative phosphorylation in the presence of functional mitochondria and oxygen availability. This metabolic plasticity though enables survival of cancer cells in turn will; (i) instigate the production of ROS. Excessive ROS levels induce mito/ER stress leading to the up-regulation of stress inducible MIC-A/B eventually increasing the sensitivity to NK cells mediated cytotoxicity. (ii) reduce the acidity of TME and prevent low-pH enabling the tumor-infiltrating immune cells to target cancer cells. Furthermore, inhibition of HIF-1 α one of the transcriptional regulators of SDF-1 could mitigate TAM activation and TAM-dependent inhibition of T cells. LDH, lactate dehydrogenase; ROS, reactive oxygen species; mito/ER stress, mitochondrial/endoplasmic reticulum stress; MIC-A/B, MHC class I chain (MIC)-related protein A or B (MIC-A/B); TME, tumor microenvironment; HIF-1 α , hypoxia inducible factor 1 α ; SDF-1, stromal cell derived factor-1; TAM- tumor-associated macrophage.

Fig.1

Figure 2

a

b

Fig. 3

a

b