Published in final edited form as:

Cancer Immunol Immunother. 2020 February ; 69(2): 255–261. doi:10.1007/s00262-019-02432-7.

The promise and peril of targeting cell metabolism for cancer therapy

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Abstract

A major challenge of cancer immunotherapy is the potential for undesirable effects on bystander cells and tumor-associated immune cells. Fundamentally, we need to understand what effect targeting tumor metabolism has upon the metabolism and phenotype of tumor-associated leukocytes, whose function can be critical for effective cancer therapeutic strategies. Undesirable effects of cancer therapeutics are a major reason for drug associated toxicity, which confounds drug dosing and efficacy. As with any chemotherapeutic agent, drugs targeting tumor metabolism will exert potent effects on host stromal cells and tumor-associated leukocytes. Any drug targeting glycolysis, for example, could metabolically starve tumor infiltrating T cells, inhibit their effector function and enable tumor progression. The targeting of oxidative phosphorylation in tumors will have complex effects on the polarization and function of tumor-associated macrophages. In short, we need to improve our understanding of tumor and immune cell metabolism and devise ways to specifically target tumors without compromising necessary host metabolism. Exploiting cell-specific metabolic pathways to directly target tumor cells may minimize detrimental effects on tumor-associated leukocytes.

Precis:

Drugs targeting tumor metabolism will exert potent effects on host and tumor cells. Itaconic acid is a macrophage-predominant metabolite that represents a viable therapeutic target for the control of inflammation and tumors.

Keywords

immunometabolism; tumor metabolism; itaconic acid; macrophages; CITIM 2019

The Promise of Targeting Cancer Metabolism

Many of the earliest anti-neoplastic drugs to be developed were those that would selectively target proliferating cells. Indeed, aberrant cell growth of tumors is associated with altered metabolism to support biosynthetic demands and nucleotide production. This led to the

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Conflict of Interest

The author declares that he has no conflict of interest.

Compliance with Ethical Standards: Not applicable

development of purine and pyrimidine analogues that would directly target DNA synthesis. Unfortunately, these are largely indiscriminate, targeting proliferating normal as well as neoplastic cells alike (Figure 1). As such, toxicity and painful side-effects remain a major concern.

Aside from altered nucleotide metabolism, tumors also have other targetable metabolic pathways. Whereas normal cells generate energy mainly by mitochondrial oxidative phosphorylation (OXPHOS), proliferating cancer cells favor aerobic glycolysis, a process referred to as the “Warburg effect”. Although aerobic glycolysis is not as efficient for the generation of ATP, it allows for production of nucleotides, amino acids and lipids, which are the necessary building blocks of new cells and tissues. Metabolic adaptation of cancer cells enables rapid cell proliferation and cell survival even in hypoxic, nutrient-depleted and inhospitable cellular environments. Hypoxia, commonly observed in tumor environments, promotes glycolysis through a HIF-1 α dependent mechanism [1]. HIF-1 α induces the expression of glucose transporters and glycolytic enzymes while simultaneously suppressing OXPHOS through the action of pyruvate dehydrogenase kinase (PDK) [2]. The induction of PDK results in a diversion of glucose towards lactate, prevention of OXPHOS, with the added effect of protecting tumor cells from OXPHOS-derived reactive oxygen species (ROS) and DNA damage [3]. Targeting the metabolism of cancer cells represents an attractive therapeutic strategy. Due to the high degree of glycolysis in tumors, drugs have been developed to target this pathway, as an attempt to deprive tumors of ATP and biosynthetic molecules required for proliferation. Glycolytic inhibitors such as 2-DG and other hexokinase inhibitors such as 3-bromopyruvate induce cell death by depriving cancer cells of glucose [4]. Inhibition of PDK is another attractive option, as targeting this enzyme within tumors would be expected to suppress tumor glycolysis and promote OXPHOS-driven ROS expression. One such PDK inhibitor, dichloroacetate, was shown to preferentially kill cancer cells in a rat model of lung cancer [5]. Unfortunately, such approaches are non-selective, and the translational potential of these glycolytic inhibitors is limited. Indeed, many immune cells such as T cells require high levels of glucose for their effector function.

In the absence of glucose, cancer cells will utilize fats and amino acids to produce ATP from OXPHOS. For this reason, another metabolic target could be mitochondrial OXPHOS. Consistently, several inhibitors of mitochondrial complex I have been utilized as cancer therapeutics. One of the most well characterized of these, metformin, inhibited tumorigenesis in *in vitro* and *in vivo* [6]. Another mitochondrial complex I inhibitor, BAY 87–2243, decreased the growth of BRAF mutant melanoma in a mouse xenograft model, in association with decreased oxygen consumption and increased generation of reactive oxygen species (ROS) [7]. Additional evidence suggests certain tumors use non-glycolytic means by which to derive cellular energy, such as fatty acid oxidation in the case of prostate cancer [8] and, in those cases, inhibitors of fatty acid synthesis show promise. An ongoing challenge of tumor therapy is to overcome the considerable heterogeneity of tumor cell metabolism that enable tumors to adapt and grow in hypoxic environments in which glucose and other nutrients are limited.

Metabolic needs of tumor-associated leukocytes

Normal cells, as well as tumor cells, adapt to specific microenvironments in their utilization of fuels for cellular metabolism. Indeed, all cells are sensitive to alterations in their microenvironment. In cancer, the competitive advantage for tumor cells to consume nutrients, such as glucose, makes a nutrient-restricted environment that is inhibitory to immune cell function. Glucose is required for glycolysis and T cell mediated anti-tumor responses. Tumor-imposed metabolic restrictions can result in hypo-responsiveness of T cells during cancer development, rendering them ineffective for anti-tumor responses [9]. Restoration of T cell glycolysis restores IFN- γ production and T cell responses to tumors. Tumors are frequently comprised of regulatory T cells which dampen immune responses and allow for tumor progression. In this regard, it is important to note that these regulatory T cells, unlike effector T cells, are not reliant on glucose, but rather rely upon fatty acid oxidation [10]. The ability to store fatty acids give regulatory T cells a metabolic advantage within the tumor microenvironment. Fatty acid oxidation is also important for the development and function of myeloid-derived suppressor cells (MDSC), a heterogeneous population of myeloid cells which inhibit anti-tumor immunity through various mechanisms including ROS [11–13]. Recently, we showed that immature neutrophils also use mitochondrial fatty acid oxidation to support ROS production and T cell suppression [14]. Poly-unsaturated fatty acids can promote the development of MDSC *in vitro* and *in vivo* [15]. Hossain *et al* demonstrated that MDSC have increased uptake of fatty acids, mitochondrial mass and oxygen consumption rates at tumor sites [16], which are necessary for immunosuppressive activities. The scavenging or depletion of amino acids critical for T cell functions underlie many of the immunosuppressive mechanisms MDSC [11, 17, 18]. Regardless of the mode of immunosuppression, the finding that the tumor microenvironment can promote the metabolic needs of regulatory T cells and MDSC while restricting those of anti-tumor T cells highlights the degree to which tumors manipulate their metabolic microenvironment to prevent optimum functioning of tumor-associated leukocytes.

Although macrophages can mediate important anti-tumor responses, there is considerable evidence for their role in promoting the initiation, growth and metastatic spread of many tumors. The ability for macrophages to produce immunosuppressive cytokines, tumor-promoting growth and angiogenic factors has been well described [reviewed in [19]]. Although the classification of macrophages along the spectrum of anti-tumor, classically-activated “M1” and pro-tumor, alternatively-activated “M2” phenotypes is somewhat simplistic, it is a useful reminder that metabolic differences can result in either a pro- or anti-inflammatory macrophage which clearly demonstrates the important linkage between metabolism and cellular function [20, 21]. M1 macrophages have increased glycolysis, which maintains high ATP levels and favors NADPH production which results in the production of nitric oxide and reactive oxygen species. Conversely, M2 macrophages generate ATP primarily through oxidative phosphorylation and fatty acid oxidation, which can be sustained for longer periods of time. Since macrophages adapt their metabolism due to alterations in their environment, the enhancement of fatty acid oxidation by tumors could help adapt tumor-associated macrophages towards the pro-tumoral M2 phenotype [20, 22].

The metabolism and function of tissue-resident macrophages is further refined by the influence of localized factors. Recent studies in the peritoneal cavity have refined our view on how resident macrophages metabolically adapt to maintain tissue homeostasis. The peritoneal cavity is rich in retinoic acid, which drives the expression of Gata-6, a transcription factor critical for the peritoneal resident macrophage phenotype [23, 24]. The peritoneal cavity is rich in metabolites such as N-acetylaspartate and glutamate, the latter of which can be utilized by highly oxidative, resident macrophages during phagocytosis to fuel mitochondrial function for enhanced ROS production [22, 25]. Resident macrophages thus utilize locally available fuels to implement metabolic responses to extracellular cues and their function is adapted to their metabolic niche.

Metabolic cross-talk between tumors and immune cells

Although tumor metabolism has been well studied, the effects of host-tumor interactions on immune cell metabolism is less well understood. This knowledge gap represents a challenge as metabolic-based therapies are deployed. As well as depriving the tumor microenvironment, tumor-derived factors can influence immune function. [9]. Lactic acid, a product of glycolysis, has been associated with the polarization of “M2” macrophages, characterized by tumor-promoting activities, such as the expression of VEGF and arginase [26]. Other mechanisms by which macrophage metabolism may be affected by tumors are highlighted by their lack of fatty acid oxidation which results in accumulation of TAM-promoting lipid droplets by the caspase-dependent cleavage of peroxisome proliferator-activated receptor gamma (PPAR- γ) [27]. Small amounts of extracellular citrate can also be incorporated into tumor cells, at physiological levels found in the blood during hypoxic glucose-starved conditions [28]. Thus, it is intriguing to speculate that activation of tumor-associated macrophages could supply a source of extracellular citrate that promotes tumor growth. There are presumably many undiscovered fuels and signals associated with tumors involved in metabolic alterations and polarization in macrophages.

As mentioned previously, the peritoneal cavity represents a distinct metastatic niche which is home to heterogeneous populations of tissue-resident macrophages that play important roles in tissue homeostasis and immune surveillance [22, 25]. There is the strong potential for extensive cross-talk between tissue-resident macrophages and tumors that develop in, or metastasize to, the peritoneal cavity. Recently, we found that peritoneal tumors increase the expression of the *Irg1* gene and itaconic acid production in resident macrophages [29]. As described later, itaconic acid is an intriguing metabolite in that it links cellular metabolism with inflammation, as well as ROS production in macrophages by regulating mitochondrial respiration and oxidative phosphorylation [30, 31]. Although the mechanisms by which tumors upregulate itaconate production in resident macrophages remains to be determined, this metabolite facilitates tumor progression in the peritoneal cavity and may represent a novel therapeutic target for the treatment of metastatic disease. It is important to note, however, that tumor-elicited changes in the metabolism of peritoneal, tissue-resident macrophages do not recapitulate every tumor microenvironment, and additional studies are urgently needed on the immunometabolism in other tumor models and environments.

Itaconate is an attractive metabolic target for cancer therapy

Immune cells must sense and respond to alterations in their extracellular environment to protect the host against pathogens. Metabolic reprogramming of cells is one of the earliest changes in response to proinflammatory stimuli. Itaconic acid (itaconate) is a classic example of a metabolite that can moonlight as an immunomodulator. Itaconate is a dicarboxylic acid that is formed from the catabolism of mitochondrial cis-aconitate, mediated by the enzymatic activity of a *cis*-aconitate decarboxylase (CadA) gene [32] (Figure 2). Early interest in itaconate stemmed from its usefulness as a building block for plastics and polymers. Since the 1960s, itaconate has been manufactured at large scales by the fermentation of *Aspergillus terreus* and, to a lesser extent, other microorganisms [33]. Considerable interest in itaconate is also owed to its anti-microbial properties, as itaconate inhibits the bacterial enzyme isocitrate lyase, an enzyme of the glyoxylate shunt used as a bacterial survival mechanism. Itaconate was recently identified as a mammalian metabolite, the product of an LPS-inducible gene within macrophages named immunoresponsive gene 1 (IRG1) that is the mammalian homolog of CadA [32, 34]. Moreover, the IRG1 gene product responsible for itaconate production is significantly upregulated in LPS-stimulated macrophages [34]. Itaconate is among the most selectively and highly upregulated metabolites in classically-activated macrophages [35]. Itaconate levels are linked to inhibition of succinate dehydrogenase (SDH), also known as complex II in the mitochondrial electron transport chain, and the accumulation of succinate, which has pleiotropic effects on downstream signaling pathways during mitochondrial respiration [30, 36, 37]. Succinate has been shown to modulate macrophage function, indicating a potential for metabolic feedback on macrophage phenotype.

Itaconate is significantly upregulated in macrophages during inflammation [35]. The dramatic upregulation of Irg1 and itaconate in response to pathogens or proinflammatory stimuli is consistent with a key role for this metabolite in the metabolic reprogramming of immune cells. In macrophages, itaconate has been shown to have anti-inflammatory effects (reviewed in [37]). One of the earliest indications for the immunomodulatory role for itaconate came from studies in which macrophages were treated *in vitro* with a cell-permeable form of itaconate which resulted in reduced expression of some key M1 macrophage proinflammatory cytokines, namely IL-6, IL-1 β and IL-12p70 [30]. Conversely, bone marrow derived macrophages from *Irg1*^{-/-} mice had increased expression of these cytokines in response to LPS or inflammasome stimulation than their wildtype counterparts. The anti-inflammatory properties of itaconic acid was initially believed to be through its ability to inhibit SDH (Figure 2) [30]. Recently, it was shown that itaconate also directly affects signal transduction. By virtue of electrophilic properties, itaconate alkylates the protein KEAP1 which normally associates with and promotes degradation of the Nrf2 transcription factor [38]. Cell-permeable forms of itaconic acid were shown to alkylate KEAP1 and enable accumulation of Nrf2, which in turn activated anti-oxidant and anti-inflammatory responses (Figure 2) [39]. Itaconate has also been implicated in negative regulation of type I interferon signaling [38]. Acting through any of these mechanisms, the upregulated itaconate expression during inflammation is associated with an attenuation of

pro-inflammatory cytokine production and ROS generation in stimulated macrophages [30, 31, 37, 40].

Although itaconate has anti-inflammatory properties in several inflammatory models involving macrophages, we showed that this metabolite also plays a direct role on tumor progression in the peritoneal cavity. In our recent study, we found that peritoneal tumors profoundly alter the metabolism of resident macrophages [29]. The presence of peritoneal tumors resulted in itaconate production by resident macrophages. Using unbiased metabolomics, we identified that itaconic acid was the most highly upregulated metabolite in peritoneal resident macrophages [29]. We showed further that the tumor-elicited increase in *Irg1* expression is important for tumor progression, since the targeting of *Irg1* by lentiviral-based gene knockdown resulted in significantly reduced tumor burdens. Intriguingly, we found increased OXPHOS in the high *Irg1* expression resident macrophages from tumor-bearing mice, a finding which is seemingly at odds with the established role for itaconate mediated inhibition of SDH. We believe this reflects the increased utilization of fatty acids to fuel OXPHOS in the tumor setting. The mechanism by which itaconate promotes tumor progression includes facilitating the beta-oxidation of fatty acids as a fuel source for driving enhanced mitochondrial OXPHOS. *Irg1* catabolizes fatty acid-derived metabolites which may serve as energy substrates for OXPHOS in macrophages and OXPHOS-associated reactive oxygen species (ROS) production by mitochondria [31, 40, 41]. Through alterations in fatty acid oxidation and OXPHOS, itaconic acid serves as a nexus between immunometabolism and inflammation. In the tumor setting, pro-tumor responses may be elicited through the pleiotropic effects of ROS, including the mitogen-activated protein kinase (MAPK) activation in tumor cells [29]. We also showed *IRG1* was highly expressed in CD14⁺ monocytes from ovarian carcinoma patient ascites, suggesting this metabolite is important in the progression of ovarian carcinoma in humans [29]. *IRG1* expression in tumor cells also correlated with glioma progression [42], further highlighting the important role this metabolic pathway might play in regulating the progression of some tumors.

Since *Irg1* is predominantly expressed in peritoneal macrophages, and not expressed by tumors, the therapeutic targeting of this metabolic pathway should lead to the selective activation of macrophages within the tumor microenvironment. Targeting itaconate thus holds promise for controlling tumors without eliciting detrimental side effects generally associated with systemic ROS or MAPK inhibition (Figure 1). Although itaconic acid expression has not been reported in T cells, it is certainly conceivable that targeting this metabolite could have indirect consequences on T cell function in the tumor microenvironment, for example through alterations in macrophage production of ROS or proinflammatory cytokines, as noted previously. Further studies on the potential effects of metabolic intervention on the development and function of multiple immune cell compartments are warranted.

Future Perspectives

To make targeting tumor metabolism an efficacious therapeutic approach, we need to understand tumor and immune cell metabolism better and devise ways to specifically target one without compromising the good/leukocyte metabolism (Figure 1). Drugs targeting

tumor metabolism will exert potent effects on host tumor-associated leukocytes, with consequences on effector function of immune cells we do not fully understand. Drugs targeting tumor glycolysis, for example, will have undesirable effects on the glycolysis of tumor-associated leukocytes which usually rely upon glycolysis to derive the cellular energy necessary for them to carry out effector functions. The identification of metabolites that play important roles during tumor growth needs to be continued, and those with cell-specific expression profiles might be exploited for generating anti-tumor responses with reduced side effects. Itaconic acid is one such attractive candidate, since specifically targeting resident macrophage-associated itaconate levels could control peritoneal tumors. Since Irg1 is predominantly expressed in macrophages, this approach might avoid side effects often observed with systemic ROS or MAPK inhibition. The identification of itaconic acid as an important regulator of resident macrophage function raises the intriguing question of how this might be utilized therapeutically for the control of ovarian and peritoneal tumors. The importance of itaconic acid as an inhibitor of metabolic pathways such as those for isocitrate lyase in bacteria and glycolysis in mammals may yield clues for its development as a therapeutic target. As summarized before in [43], certain pathogenic gram-negative bacteria, such as *Yersinia pestis* and *Pseudomonas aeruginosa*, degrade itaconate as a survival mechanism by converting it into pyruvate and acetyl Co-A (Figure 2) [32, 44]. Mammals have similar mechanisms for degrading itaconate [45, 46] that are probably needed to counteract the itaconate-mediated inhibition of glycolysis [47]. There may even be natural products capable of binding Irg1 and inhibiting itaconate production. Additional research is needed so that the targeting of itaconate for degradation or inhibition becomes a practical means by which peritoneal tumors and perhaps inflammatory diseases can be treated.

As a broader perspective, Irg1 and itaconate may be a component of MDSC suppressor function, and itaconate may play an important role in the immunosuppression associated with many different types of cancer. Irg1 has been implicated in the fatty acid beta-oxidation, a process which is central to the suppressive properties of MDSC [12, 16]. It would be interesting to study metabolic changes in MDSC and determine whether targeting itaconic acid impacts MDSC-mediated immunosuppression. It could be very important to look at the regulation and expression of itaconic acid in distinct myeloid cell and anatomical compartments during cancer and inflammation.

Funding:

This work was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, Cancer and Inflammation Program.

List of abbreviations:

HIF-1a	hypoxia induced factor 1 alpha
Irg1	immune responsive gene 1
NADPH	nicotinamide adenine dinucleotide phosphate
OXPHOS	oxidative phosphorylation

ROS	reactive oxygen species
SDH	succinate dehydrogenase

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Figure 1. Metabolic-based anti-cancer strategies need to consider the potential for unanticipated or undesirable effects on tumor-associated leukocytes.

Inhibitors of glycolysis, for example, will likely impair effector T cells which rely upon glycolysis. Drugs targeting OXPHOS in tumors may alter the polarization and function of tumor-associated macrophages. The identification of itaconic acid as a pro-tumor metabolite that is preferentially expressed by tumor-associated macrophages, suggests targeting this metabolite in the context of peritoneal tumors could lead to anti-tumor responses that are not associated with undesired effects on other cells.

Figure 2. Formation and degradation of itaconic acid.

Itaconic acid is a dicarboxylic acid formed by the decarboxylation of cis-aconitate, a component of the Citric Acid Cycle. This reaction is mediated by the gene product of bacterial Acod1 (aconitate decarboxylase 1) or mammalian Irg1 (immune-responsive gene 1). Itaconic acid mediates immunosuppression by multiple pathways (reviewed in [37]). It inhibits succinate dehydrogenase (SDH), resulting in succinate accumulation and suppression of ROS and proinflammatory cytokine expression upon cell activation. Itaconic acid also activates NRF2 which may also reduce ROS and IL-1 β expression. The breakdown or catabolism of itaconic acid involves its conversion into itaconyl-CoA by Ict (itaconate CoA transferase) or succinate-CoA ligase and further processing into pyruvate and acetyl-CoA [32, 46].