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Reprogram Metabolism of Infiltrated T Cells in Tumor Microenvironment to Improve Immunotherapy Outcomes

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Abstract

Increasing clinical evidences demonstrated that the immune system involved in antigen recognition and cytokine production events may inhibit tumor initiation and progression. Actually, tumors undergoing remission in immunotherapy are largely attributed to Tumor-Infiltrating Lymphocytes (TILs). The quantity, functionality, durability and longevity of TILs harnessed for anti-tumor activity could be important determinants in achieving the efficacy of therapy. The persistence of metabolic qualities of highly-functional T cells is the key to improve immunotherapy outcomes with favorable prognosis. In this review, we focus on metabolic properties of TILs including cellular energetic pathway and discuss the potential approaches and detailed strategies to meticulously modulate infiltrated T cells. Researching and developing chemotherapy drugs armed with immune-enhancing power could transform the medical territory towards cancer.

Keywords: Combination Therapy; Immuno-metabolism; Metabolic Flux; Tumor-Infiltrating Lymphocytes

Abbreviations

TILs	:	Tumor-Infiltrating Lymphocytes
TCRs	:	T Cell Receptors
IFN-γ	:	Interferon Gamma
TCA	:	Tricarboxylic Acid
OXPHOS	:	Oxidative Phosphorylation
mTOR	:	Mammalian Target of Rapamycin
PI3K	:	Phosphatidylinsitol 3-Kinase
NHL	:	Non-Hodgkin Lymphoma
AMPK	:	Adenosine Monophosphate-Activated Protein Kinase
CTLs	:	Cytotoxic T Lymphocytes
SRC	:	Spare Respiratory Capacity
ROS	:	Reactive Oxygen Species
HIF1α	:	Hypoxia Inducible Factor 1α
VHL	:	Von Hipple-Lindau
MDSC	:	Myeloid-Derived Suppressor Cells
DCs	:	Dendritic Cells
IDO	:	Indoleamine 2,3-Dioxygenase
COX2	:	Cyclooxygenase
MAPK	:	Mitogen-Activated Protein Kinase

Background

Since more than 150 years ago, Rudolf Virchow proposed the relationship between immune function and cancer when he observed the prevalence of leukocytes in tumors [1], and subsequent Friedrich Fehleisen and William Corley reported the scientific attempt to determine the nature and limits of the treatment for sarcoma by inoculation with erysipelas in late19th century [2], immunotherapy towards cancer is a continuous standing subject important enough all the time. Especially recent regulatory approval of ipilimumab (Yervoy; Bristol-Myers Squibb), pembrolizumab (Keytruda; Merck) and novolumab (Opidivo; Bristol-Myers Squibb) et al, and the dendritic cell therapy sipuleucel-T (Provenge; Dendreon) as well as the Chimeric Antigen Receptors (CAR)-T therapy tisagenlecleucel-T (Kymriah; Novartis) evince that the immune

system could be modulated to provide a reproducible benefit for patients suffering cancer [3-6].

These clinical successes for cancer treatment implies the interpretation how immune metabolism supports many aspects of cellular function and how metabolic reprogramming can drive cell differentiation and fate [7-10]. However, it is not yet clear to what degree immunotherapy will benefits patients with other types of cancer besides the authorized indications such as melanoma, acute lymphoblastic leukemia, synovial sarcoma, and lung cancer et al. [11-13]. In this review, we combs through the metabolism of Tumor-Infiltrating Lymphocytes (TILs) for the scientific strategies to improve the therapy outcomes by analyzing metabolic configuration resulted from the heterogeneous rate of metabolic conversion in the cellular network.

Principles of Immuno-Metabolism Modulation to Treat Human Cancer

Cancer immunotherapy is proposed on the clinical observations and idea that, as a result of unique mutations or protein expression patterns, tumor cells with neoantigen may be recognized and eliminated by the immune system with discriminating between self and non-self and a high degree of specificity [14,15]. Besides the other several forms currently under try in immunotherapy [16], present therapeutic approaches that seek to utilize T cells to paralyze tumors can mainly be divided into three categories:

1) Genetic modification of T cells to confer the ability to specifically recognize malignant cells, as exemplified the CARs and tumor antigen-specific T Cell Receptors (TCRs) transduction therapy [17,18],

2) Isolation of tumor-specific T cells from existing tumor masses, followed by *ex vivo* expansion and reinfusion into the patient, known as Adoptive Cell Transfer (ACT) of TILs [19,20],

3) Treatment of patients with agents designed to activate tumor-specific T cell *in situ* [21-23], actually involved partly in the immune metabolism modulation.

Immuno-metabolism, the interplay between immunology and metabolism, especially focuses on the emerging role of the intracellular metabolic machinery in the regulation of an immune response. The insights of intracellular metabolic pathways controlling immune responses helps us understand the bio-energetic requirements of T cell differentiation, cell-fate decision and function of T cells [24,25]. Metabolic changes such as nutrition deprivation or overload could dictate the characteristics of the T cell compartment and subsequent downstream immune reactions [26]. Current methods for generating T cell products for adoptive immunotherapy have the pitfall of driving cells toward terminal differentiation and senescence [27,28]. Approaches that are informed by a deepened knowledge the metabolic requirements for the optimal function of anti-tumor T cells are likely to be the subject of intense work in the field of immune metabolism [29].

TILs in Tumor Microenvironment

Tumors are now recognized as structures of multiple cell types [30]. Emerging evidence indicates that to effectively control cancer, we need to consider carcinogenesis and tumor progression not as a cell autonomous, cancer-cell centered condition, but rather as disease involving complex heterotypic multi-cellular interactions within a newly formed tissue [31]. In addition to malignant cells, the tumor microenvironment also includes nonmalignant cells including TILs, endothelial cells, pericytes, smooth muscle cells, fibroblasts, carcinoma-associated fibroblasts, neutrophils, basophils, mast cells, B lymphocytes, natural killer cells and Antigen Presenting Cells (APC) such as macrophages and dendritic cells [32], secreted proteins, and blood vessels that surround and support the growth of the tumor. The structure and composition of the tumor microenvironment varies among different types of cancers and between patients [33]. Furthermore, even among patients with the same type of cancer, the frequency, distribution and function of immune cells may differ, and this inter-individual variability has been showing to affect patient outcome [34,35]. Tumors biopsies from patients diagnosed with B-cell lymphomas are typically characterized by a high prevalence of T cells with the tumor microenvironment, with T cells comprising up to 50% of the intratumoral cells [36].

Tumor microenvironment is characterized by a consistent reduction in oxygen and blood-borne nutrients that significantly affects the metabolism of distinct cell subsets [37]. It is increasingly appreciated that as the neoplasm initiates and progresses, the surrounding microenvironment coevolving through continuous paracrine communication and supporting carcinogenesis is activated [38]. The cancer cells can themselves manipulate the microenvironment by skewing the differentiation of infiltrated T cells, attracting regulatory T cells or suppressive monocytes, or secreting immunosuppressive cytokines [39,40].

TILs can be divided into two groups: those expressing CD4 and those expressing CD8. Those cells are both responsible for the performance of anti-cancer activity. However, the type of $CD8^+$ cells is named as cytotoxic T cells in which expressing the FasL in the surface and they have the capability to kill and disrupt the target cells by secreting interferon gamma (IFN-γ), granzyme B and perforin which endow cytotoxic and apoptotic activity for those cells [41]. In the other hand, $CD4^+$ T cells can enhance the production of antibodies by neighboring B cells, and promote an effective immune response through the production of cytokines such as IL-2 and chemokines [42]. The patients having the higher amount of TILs were shown that the tend to exhibit better prognosis in various types of solid cancer including breast, lung, colon, pancreatic and hematologic malignancies et al. [43-45]. However,

the quantity and activity of TILs can be affected by several factors encompassing the trafficking hurdle, the amount of cytokines or chemokines which are released by the tumor microenvironment, the behavior of interactions between TCR and MHC molecule, as well as the metabolic configuration [46,47].

Increasing Quantity of T Cells Trafficked and Infiltrated into Tumors

Regardless of ACT therapy and CAR-T, it is obvious that pharmacological enhancement of quantity of T cells recruited, trafficked and infiltrated into tumors can provide solutions for the optimization of therapeutic option in cancer immunotherapy. Compared to the B-cell Non-Hodgkin Lymphoma (NHL) in which there is abundant immune cells in secondary lymphoid organs, infiltration of immune cells is more limited in solid tumors [48]. Therefore, the goal in this segment is to induce more population of T cells. The detailed illustration for naïve T cell migrating through specialized endothelium of secondary lymphoid and ways as well as the mechanisms to enhance T cells trafficking into tumors by infiltrating through post-capillary venules into the target tissue to their antigenic site have been covered in other reviews [49-53], and therefore we will not discuss in detail in this review.

Favoring the Functionality of Existed TILs at the Tumor Site

Experiencing many potential obstacles to reach the tumor site, TILs encountered metabolic alterations within the tumor mass in which also limit T cell functions. Restoring and favoring the anti-tumor functionality of TILs could render the significant improvement of therapeutic effectiveness.

Metabolism which is integrated into every cellular process and fate decision controls T-cell lineage choices. In regard of this aspect, Ericka L.Pearce from Max Planck Institute in Germany and Nicholas P. Restifo from National Institute of Health in USA et al. as the representatives proposed their excellent opinions in the recent several intensive published reviews [10,54,55]. Manipulation of immunometabolism has emerged from these related studies. Simply stated, different immune cell functions are associated with distinct metabolic configurations. For example, resting immune cells utilize energetically efficient processes such as the Tricarboxylic Acid (TCA), linked to the generation of ATP via Oxidative Phosphorylation (OXPHOS), while activated T cells use aerobic glycolysis [56]. Novel metabolic features and metabolism-regulated function in TILs have recently been identified and could be targeted therapeutically by modification of system metabolism [57].

As described in the reviews [58-60], adaption of specific metabolic phenotype is critical for TILs immune functions. A key metabolic regulator of infiltrated T cells is Mammalian Target of Rapamycin (mTOR), which integrates immune and metabolic cues, such as antigens via PI3K-Akt signaling and nutrients via amino acid sensing, and as an intracellular kinase phosphorylates downstream targets to mediate mRNA translation and protein degradation [61]. To be deserved to mention, rapamycin, the inhibitor of mTOR and as an immunosuppressive drug, was effective during both the expansion and contraction phases of the T cell response. During the expansion phase rapamycin increased the number of memory precursors and during the contraction phase which means effector to memory transition it accelerated the memory T cell differentiation program [62]. Proteins associated with mitochondrial OXPHOS and glutamine metabolism were significantly increased following rapamycin treatment, indicating selectivity of the role of mTORC1 in T cell metabolism. [63]. This mTOR pathway directs T cell metabolic reprogramming and ultimately acquisition of effect function, as reported that pharmacological or siRNA targeting of mTORC1 can increase memory CD+ T cell response in tumor models [64]. It also needs to keep in mind that the use of mTOR to modulate metabolism in TILs in right doses of its inhibitor, treatment timing, and/or tumor characteristics [65,66] to avoid the contrary outcomes.

Another regulator adenosine Monophosphate-Activated Protein Kinase (AMPK)'s roles which counterbalances mTOR in T lymphocytes energy metabolism homeostasis have also been revealed in recent years [67,68]. AMPK is an evolutionarily conserved serine/theronine kinsase that modulate the cellular response to an energy challenge. AMPK activation is regulated by the cellular AMP/ATP ratio and by upstream kinases [69]. Once activated by stimuli like muscle contraction, hypoxia, metabolic poisoning, inflammation or sepsis, the kinase switches on ATP-producing catabolic pathways and switches off ATP-consuming anabolic process, via both direct phosphorylation of regulatory proteins such as PFK2 involved in glycolysis and Acetyl-CoA carboxylase involved in fatty acid synthesis and indirect effects on gene expression [69]. Differentiation of naïve T cells to effector cells and subsequent long-term memory cells is tightly associated with changes in their energy metabolic activity, and therefore fine-tuning of metabolism by AMPK could modulate TILs functions [70].

Together with other regulators such as PIM2 and AKT in Phosphatidylinsitol 3-Kinase (PI3K) pathway for TILs functionality [71], metabolism thus represents a key node of regulation for T cell fate decision. Sufficient and sometimes distinct fuels are required throughout their functional states as naïve, effector or memory T cells. T cells can enter into alternative, dysfunctional states when these needs are not met, including energy referred as metabolic inert and exhaustion meaning metabolic sufficient. Future illustration of how metabolic fitness underlies healthy and dysfunctional T cells has the potential to discover novel therapeutic modalities for the immunotherapy [72,73].

Enhancing the Persistence of Activated TILs

Activated TILs could exhibit anti-tumor effect via producing effector molecules and inducing tumor cell apoptosis. However, persistence is the paramount intrinsic quality for activated TILs with anti-tumor engagement. As recorded by high-resolution 4D imaging [74], Cytotoxic T Lymphocytes (CTLs) use polarized secretion accompanied with temporal order of events to rapidly destroy tumor cells. In the adoptive transfer of TILs setting, T cells in human patients that mount effective anti-tumor responses are characterized by increased persistence and survival *in vivo* [75]. Cells with early differentiation states, including naïve T cells, stem central memory T cells, and central memory T cells, are more efficacious in treating established vascularized melanoma than terminally differentiated effector memory T cells or effector T cells, suggesting that acquisition of full effector function *in vitro* paradoxically compromise the *in vivo* antitumor efficacy of adoptively transferred $CD8^+$ T cells [76]. Interestingly, positive correlations between the expression of markers along with telomere length in T cells and the magnitude of response against tumors have been found [77].

Recent emerging evidences demonstrated that metabolic interventions can provide a therapeutic strategy to enhance lifespan of TILs [78]. Usually, increased metabolic activity alterations drive rapid proliferation of activated T lymphocytes but can also results in decreased cell persistence and increased susceptibility to cell death [79]. The condition is similar as the adage saying "you can't have your cake and eat it". Several attempts have been conducted to promote increased longevity of TILs. Genetic deletion of Atg5 or Atg7 playing roles in catabolic autophagy results in impaired memory cell formation and long-term survival of $CD8^+$ cells [80]. In addition, inhibition of mTORC1 and T cell glycolysis, as well as activation of AMPK signaling pathway, can promote TILs persistence *in vivo* besides the functionality mentioned above [81-83]. It has been indicated that T cells that take up low levels of glucose are metabolically fit for long-term survival and memory cells formation [82]. In further, the role fatty acid oxidation and mitochondrial metabolism in the promotion of T cell persistence and longevity also been explored. By measuring upon treatment of cells with the uncoupled agent FCCP, increased mitochondrial Spare Respiratory Capacity (SRC) is revealed to exhibit increased longevity and persistence of T cells [84]. However, stabilization of Hypoxia Inducible Factor 1α (HIF1α) through the loss of tumor suppressor Von Hipple-Lindau (VHL) results in T cells with increased *in vivo* persistence, simultaneously along with elevated glycolysis and no increase in SRC [85]. Meanwhile, T cells with increased mitochondrial membrane potential (Δφ), which is indicative of high rates of glycolysis and basal mitochondrial oxidative metabolism and associated with increased Reactive Oxygen Species (ROS) production and DNA damage, have reduced persistence and self-renewal capacity compared with cell exhibiting low Δφ [86]. Take together, moderate metabolic activity other than high rates of metabolic activity benefits the persistence and longevity of TILs, and overall rate of cellular metabolic activity, rather that of specific pathways is deserved to be systemically considered to enhancing the persistence of activated TILs.

Overcoming the Suppressive Constraint Over TILs

Unfortunately, TILs as the potential soldier fighting with the enemy cancer is kidnapped in the tumor environment. Many suppressive constraints imposed on TILs release full potentiality. The success of CTLA and PD-1 antibody treatment underlying the mechanism of removal of the inhibitory signal proved this concept [3-6]. Meanwhile, TILs face a fierce competition environment for nutrients availability. For in-depth discussion, we recommend several recent reviews [87].

Simply stated, suppression of tumor-specific T cells is orchestrated by the activity of a variety of stromal myeloid and lymphoid cells, and these suppressive mechanisms are often inducible. The more the tumor comes under attack, the more counter-regulatory mechanism may be induced. For example, T cells attempting to activate in the tumor microenvironment may encounter constitutive expressions of negative regulators such as PD-1, CTLA4, Indoleamine 2,3-Dioxygenase (IDO) in the tumor cells. Myeloid-Derived Suppressor Cells (MDSC) in the tumor may generate immunosuppressive nitric oxide, arginase-1 or ROS. Tumor associated microphages may produce TGFß and VEGF, which can be inhibitory for both TILs and Dendritic Cells (DCs) [88,89]. Activated Tregs which derived from $CD4^+$ T cells can produce IL-10 and TGFß, which may directly suppress $CD8^+$ T cells. Tregs may also inhibit expression of co-stimulatory ligands CD80 and CD86 on local DCs, thus rendering them ineffective and tolerating antigen-presenting cells. As effector T cells attempt to activate, their production of IFNγ and other pro-inflammatory cytokines such as IL-2 may actively up-regulate expression of IDO and PD-L1 by DCs, thus eliciting counter-regulatory suppression. Many tumor cells may also up-regulate numerous inhibitory molecules such as IDO, PD-Ll, when responding to IFNγ [90]. Furthermore, the metabolites of Cyclooxygenase (COX2) which is over-expressed in some tumors such as colorectal cancer due to the hypoxic tumor microenvironment, primarily prostaglandin E_2, have direct effects on immune-mediated tumor escape and enhance the activity of multiple immune suppressor cells, including tumor associated macrophages, Treg cells and MDSCs [91]. Therefore, it deserved to emphasize that IDO1 and COX2 inhibitors, as well as Mitogen-Activated Protein Kinase (MAPK) inhibition and cholesterol modulation, could extend the possibilities for overcoming the suppressive constraint over TILs [92-95].

It is naturally wondered that the combination approaches which weaken as much as possible the demonstrated suppressive factors imposed over TILs would works or not. For this aspect in

further discussion, see several recent reviews [95-98, 2017]. In fact, the recent clinical trials present that PL-1 and/or CTLA-4 antibody combined with other agents based on distinct mechanisms benefits significantly the patients, though the ability of immuno-oncology to meet its full potential will depend on overcoming development challenges, including the need for clear strategies to determine optimal dose and scheduling for combination approaches [99]. It is expected that combination therapy would dominate the immunotherapy against cancer in the coming years.

Perspective

There are a lot of things to do in the coming days in the field of immune metabolism against cancer. Firstly, the mechanism that shapes the weakness of TILs in the hostile tumor microenvironment need to be completely demonstrated in light of the edging technology such as eryo-electron microscopy and immune photonics. Secondly, the quantificational metabolic flux analysis in the distinct system levels including organelle mitochondrial, T cell itself, and tumor tissue need to be strengthened. These investigations could provide systemically the cues of metabolic configuration. Targeting these metabolic configurations by specific anaplerotic reaction can effectively modulate the metabolic direction of tendency in the disturbed equilibrium. In this analysis, mining big data from -omics modalities in real-world patients' samples could facilitate this practice. Once the nature of metabolic configuration was captured when TILs become exhausted, negative amplification of TILs metabolic equilibriums towards functionality for anti-tumor activity could be practical. Thirdly, the need of more precise predictive biomarker in immunotherapy for TILs reprogramming remains pressing. Challenge accompanied with personalized immunotherapy includes demonstrating large-scale efficacy as well as cost-effective implementation of such strategies in the clinical setting. Improving immunotherapy should incorporate the genetic and non-genetic predictors of response. Establishment of such a paradigm will reveal individual immunotherapeutic susceptibilities and help tailor immunotherapy to specific genetic profiles. Last but not least, detailed clinical evaluations, such as relationship between human T cell differentiation status and anti-tumor efficacy, need to be conducted in clinical trial settings.

Conclusion

As said by Mark Twain, part of the secret of success in life is to eat what you like and let the food fight it out inside. Simply stated, we are what we consume in the process of eating and respiration. The similar principle holds true at the cellular level to support and reinvigorate TILs functionality, durability and longevity. Deepened perceiving of TILs metabolism, including the origin, development, and transition between Naïve, activated and exhausted/memory phase, till apoptosis, oncolysis and/or pyroptosis, could identify the appropriate strategies targeting its metabolic program for immunotherapy. The breakthrough success of several mAbs for PD-1 et al. leads the immunotherapy as a continuous hot topic. It is not wonder that study on immune metabolism will be of paramount important. Thus it need cross-disciplinary experts including biologist, informatician and the clinical physician working integrally to usher the new era of immunotherapy which could be the first choice to eradicate cancer.

Author's Contributions

LN, ZSS, KYY, LXL contributed to the conceptualization and writing of the draft. WLY guided as the director and revised carefully the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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