Starved and Asphyxiated: How Can CD8+ T Cells within a Tumor Microenvironment Prevent Tumor Progression

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Although cancer immunotherapy has achieved significant breakthroughs in recent years, its overall efficacy remains limited in the majority of patients. One major barrier is exhaustion of tumor antigen-specific CD8+ tumor-infiltrating lymphocytes (TILs), which conventionally has been attributed to persistent stimulation with antigen within the tumor microenvironment (TME). A series of recent studies have highlighted that the TME poses significant metabolic challenges to TILs, which may contribute to their functional exhaustion. Hypoxia increases the expression of coinhibitors on activated CD8+ T cells, which in general reduces the T cells' effector functions. It also impairs the cells' ability to gain energy through oxidative phosphorylation. Glucose limitation increases the expression of programed cell death protein-1 and reduces functions of activated CD8+ T cells. A combination of hypoxia and hypoglycemia, as is common in solid tumors, places CD8+ TILs at dual metabolic jeopardy by affecting both major pathways of energy production. Recently, a number of studies addressed the effects of metabolic stress on modulating CD8+ T cell metabolism, differentiation, and functions. Here, we discuss recent findings on how different types of metabolic stress within the TME shape the tumor-killing capacity of CD8+ T cells. We propose that manipulating the metabolism of TILs to more efficiently utilize nutrients, especially during intermittent periods of hypoxia could maximize their performance, prolong their survival and improve the efficacy of active cancer immunotherapy.

Keywords: lack of glucose, hypoxia, CD8+ T cell metabolism, metabolic stress, tumor-infiltrating lymphocytes, tumor microenvironment, fatty acid metabolism, ketone bodies

INTRODUCTION

Following antigenic stimulation, differentiation of naive CD8+ T cells into effector cells is accompanied by metabolic reprograming to accommodate their increased demand for energy and biomass formation. Resting CD8+ T cells primarily gain energy through oxidative phosphorylation (OXPHOS), the mitochondrial pathway of energy production (1). The tricarboxylic acid (TCA) cycle, which is linked to OXPHOS, oxidizes acetyl-CoA. This metabolite can be derived from carbohydrates, amino acids, or FAs. Upon CD8+ T cell activation, T cell receptor (TCR) and costimulator CD28 ligation activate the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin

(mTOR) pathways, which in turn increase the activity of hypoxia-inducible factor (HIF)-1α and Myc (2). HIF-1α augments surface expression of Glut1, and thereby allows for increased uptake of glucose. Akt and Myc increase the activity of several glycolytic enzymes, whereas PI3K activation reduces expression of enzymes of the TCA cycle and OXPHOS (3–5). All of these signals drive CD8^{+} T cells to rely increasingly on glycolysis after activation (6). Glycolysis is less efficient but faster in generating ATP and provides building blocks to support cell proliferation and effector functions.

Once T cells are past the acute activation phase, they resume energy production through the TCA cycle and OXPHOS, which is partially supported by fatty acid oxidation (FAO) (7, 8). One facilitator of this metabolic switch is increased expression of PD-1, a coinhibitor that blocks CD28-mediated activation of the mTOR pathway (9) and reduces glycolysis and promotes lipid metabolism (10). PD-1 thereby prevents overwhelming CD8^{+} T cell activities and promotes memory formation (11).

Increased expression of PD-1 also identifies the so-called exhausted CD8^{+} T cells, which evolve during chronic infections due to continuous antigen-driven stimulation (12). Exhausted CD8^{+} T cells show progressive increases in the expression of additional coinhibitors, such as lymphocyte-activation gene 3 (LAG-3), CD160, 2B4, and others, which combined with loss of effector functions eventually lead to cell death (13). Within the tumor microenvironment (TME), CD8^{+} T cells become exhausted (14). It is assumed that lack of an effective antitumor T cell response is at least in part linked to exhaustion. Consequently, blockade of coinhibitor pathways by antibodies such as to anti-PD-1 or anti-PD-L1 has yielded promising results in some cancer patients (15).

The etiology of T cell exhaustion within the TME warrants further discussion. High level of antigen as found during chronic viral infections may not be solely responsible. The amount of antigens within a tumor would not be expected to be overwhelming, especially as tumor cells commonly downregulate MHC class I expression (16), which makes their antigens virtually invisible to CD8^{+} T cells. Instead, we propose that CD8^{+} T cell exhaustion may be triggered by metabolic stress within the TME. Cancer cells ferociously consume glucose to fuel energy production through glycolysis, which can lead to hypoglycemia within tumors. In addition, angiogenesis often lags behind expansion of solid tumors, leading to hypoxia in some areas of the TME. T cells that infiltrate solid tumors thus face dual metabolic jeopardy; lack of glucose prohibits energy production through glycolysis, whereas lack of O2 prevents energy production through OXPHOS. How tumor-specific CD8^{+} tumor-infiltrating lymphocytes (TILs) cope with these challenges and how metabolic reprograming of CD8^{+} TILs will affect their antitumor performance require further investigations, in order to improve the efficacy of cancer immunotherapy.

EFFECTS OF HYPOXIA ON CD8^{+} TIL METABOLISM AND FUNCTIONS

Solid tumors commonly have areas of hypoxia. This can be caused by lack of perfusion due to structural and functional abnormalities of the tumor microvasculature, general anemia of the patient, or insufficient diffusion due to lack of angiogenesis. The latter affects cells once they are more than 70 μm away from a blood vessel. Studies have shown that up to 50–60% of solid tumors of a variety of different types possess unevenly distributed areas of hypoxia (17).

Tumor-infiltrating CD8^{+} T cells are initially activated under physiological O2 tension in peripheral lymphatic tissues. Upon tumor entry, CD8^{+} T cells will be subjected to increasingly severe hypoxia once they leave areas close to blood vessels; this will activate HIF-1α. HIF-1α signaling adjusts the cells' metabolism to allow for energy production when O2 is limiting. HIF-1α enhances glycolysis by CD8^{+} T cells mainly by promoting the activity of lactate dehydrogenase A (LDHa), while inhibits OXHPOS by increasing expression of pyruvate dehydrogenase kinase 1 (PDK1), which prevents the oxidation of pyruvate to acetyl-CoA (18, 19). CD8^{+} TILs under hypoxia therefore must increase glucose consumption to fuel glycolysis.

A series of in vitro and in vivo studies in the past two decades show that hypoxia dampens lymphocyte activation, diminishes their proliferation, and reduces the ability of activated T cells to produce cytokines or lytic enzymes (20–24). T cell activation causes release of Ca^{2+} from intracellular stores followed by sustained Ca^{2+} influx, which is inhibited by increased HIF-1α activity (25). Whole body hypoxia dampens inflammation and T cell functions in mice and humans (26, 27). These data show that hypoxia is immunosuppressive and metabolic reprograming due to increased activity of HIF-1α may contribute to reductions of immune responses. This could be caused by reduced ATP production due to impaired OXPHOS under hypoxia. Additionally, hypoxia is known to increase accumulation of reactive oxygen species (ROS), which may induce apoptosis of activated T cells (28, 29). Vice versa, activated CD8^{+} T cells with a partial deficiency in HIF-1α show enhanced production of cytokines (30, 31). One study has shown that under hypoxia or with increased HIF-1α activity T cells increase expression of coinhibitors, including CTLA-4, CD244, and LAG-3, and decrease levels of T-bet (32), a key transcription factor that controls many of the T cells' functions, again indicating that hypoxia is immunosuppressive.

Conversely, two recent reports show that hypoxia and increases in HIF-1α activity promote effector T cell functions, especially production of the lytic enzymes granzyme B and perforin (32, 33). There is a caveat with these studies; both used a protocol in which after an initial 48 h of activation, CD8^{+} T cells were rested for several days in IL-2-supplemented medium before being subjected to hypoxia. Unlike highly activated CD8^{+} T cells, resting CD8^{+} T cells rely more on FAO and OXPHOS for energy production. This metabolic reprograming and their decreased energy demand may allow CD8^{+} T cells to improve some functions upon hypoxia. In real life CD8^{+} T cells induced by a cancer vaccine or tumor antigens (TAs) that leaked into lymphatic tissues are unlikely to rest before they infiltrate a tumor, where they may receive additional activation signals. Results obtained with resting cells are thus not pertinent to TILs exposed to hypoxia. The same papers showed that genetic depletion of HIF-1α reduces CD8^{+} T cell functions while its constitutive overexpression through functional depletion of the Von Hippel–Lindau (VHL)

factor improves functions. During the initial phase of T cell activation, HIF-1α is essential to allow T cells to use glycolysis. The effects of its complete absence or increased expression during this critical phase of differentiation may differ from changes in HIF-1α activity during later phases of activation. These studies thus give limited insights into the effect of hypoxia or HIF-1α on CD8+ TILs, which encounter limited O_2 after activation in the periphery once they penetrate into the tumor.

Hypoxia and increased HIF-1α activity in tumor tissues in general correlate with poor prognosis of cancer patients (34, 35). Hypoxia not only affects protective immune responses but also promotes tumorigenesis by enhancing proliferation of cancer cells and increasing their PD-L1 surface expression (36). The latter in turn may further dampen functions and survival of PD-1+ TILs. Hypoxia may also increase the suppressive activity of tumor-infiltrating myeloid suppressor cells and tumor-associated macrophages, which will lead to further impairments of CD8+ TIL functions (37, 38). Overall, all of these studies strongly suggest that lack of O_2 negatively affects metabolism and functions of CD8+ TILs.

EFFECTS OF HYPOGLYCEMIA ON METABOLISM AND FUNCTIONS OF TILs

Glucose is crucial during the initial stages of CD8+ T cell activation. Naive CD8+ T cells can differentiate into effectors in absence of glucose but then become functionally impaired (39). Lack of glucose also dampens effector functions of fully activated CD8+ T cells both *in vitro* and *in vivo* (39–44).

Attracted by chemokines, activated CD8+ T cells regardless of their antigen specificity infiltrate solid tumors. Here, they encounter an environment where key nutrients such as glucose may be limiting due to its consumption by tumor cells (45). Although activated CD8+ T cells express increased levels of the glucose transporter Glut1, *in vitro* studies show that their effort to take up glucose is thwarted by tumor cells, which are simply more effective at consuming this key nutrient (39). CD8+ T cell glycolysis within TME may further be reduced by accumulating concentrations of tumor cell-derived lactate, which prevents the monocarboxylate transporter-1-mediated, gradient-dependent export of lactate from CD8+ T cells. Increasing concentration of lactate within CD8+ T cells in turn causes a fall in pH, which inhibits the activity of phosphofructokinase, a key enzyme of glycolysis (46). In addition, glucose deprivation increases coinhibitor PD-1 expression on activated CD8+ T cells (47), which can further reduce glycolysis but enhance FA metabolism. Blockade of PD-1 has been shown to lessen the CD8+ TILs' metabolic stress by augmenting their glycolytic capacity through increased mTOR signaling (39).

It has been reported that FAO can maintain the survival of cancer cells when glucose is not available (48). T cells may also be able to cope with lack of glucose by enhancing other metabolic pathways. Sudden deprivation of glucose can lead to drops in ATP with enhanced AMP in activated CD8+ T cells. The increased AMP:ATP ratio activates the energy sensor AMP-activated protein kinase (AMPK). AMPK is a key regulator that

reduces the T cells' energy expenditure by blocking production of cytokines (49). Furthermore, AMPK maintains T cell viability by decreasing glycolysis and anabolic processes through inhibition of the mTOR pathway, while enhancing OXPHOS fueled by FAs and glutamine (50, 51). In agreement, the studies showed that knockout of AMPK increases apoptosis of T cells activated with limited access to glucose (49).

To what degree CD8+ TILs' functions are impaired by lack of glucose within the TME may depend on the T cells' differentiation status, or, in other words, on their metabolic programing prior to enter the tumors. Recently activated CD8+ effector T cells conditioned to use glycolysis are likely most susceptible to sudden loss of exogenous glucose (52, 53), as compensatory endogenous production of glucose through gluconeogenesis or glycogen degradation are not sustainable (54). By contrast, CD8+ T cells programed to use other nutrients may cope better with restricted glucose access (55). This in turn invites the testing of metabolic drugs that reprogram T cell metabolism as adjuvant treatments for active cancer immunotherapy or adoptively transferred TA-specific T cells.

EFFECT OF HYPOXIA COMBINED WITH HYPOGLYCEMIA ON CD8+ TIL FUNCTIONS AND METABOLISM

Hypoxia inhibits OXPHOS but allows cells to gain energy through glycolysis. On the other hand, hypoglycemia reduces glycolysis but cells can switch to OXPHOS by burning other nutrients. The problem is that many tumors have low levels of glucose combined with areas of hypoxia, which foils both pathways of energy production.

Malignant cancer cells increase lipogenesis, lipolysis, FA secretion, and recruit adipose progenitors to the TME (56–58). In addition, dying tumor cells may release FAs. FAs provide ample energy through peroxisomal or mitochondrial FAO, which may be used by CD8+ TILs. FAO is preferred by some effector T cells such as those participating in graft versus host disease (59), suggesting that FAO may be preferred by T cells that encounter large quantities of antigens (60). Energy production through FAs requires more O_2 than energy production through glucose to generate equivalent amounts of ATP. OXPHOS fueled by glucose yields 36 molecules of ATP and consumes 6 molecules of O_2. By contrast, OXPHOS fueled by palmitate, a 16-carbon FA, results in a net yield of 129 ATP and requires 31 molecules of O_2. FAs thus require 1.44 times more O_2 than glucose to provide the same amount of energy, which makes it an inefficient fuel within an O_2-deprived TME.

Ketone bodies, i.e., acetoacetate, acetone, and β-hydroxybutyrate, are produced during FA catabolism when the amount of acetyl-CoA produced by FAO overwhelms the processing capacity of the TCA cycle. When acetyl-CoA declines, ketone bodies can be converted back to acetyl-CoA providing a ready source of energy that requires less O_2 than catabolism of FAs (61). Previous studies showed that under conditions of hypoxia and hypoglycemia, cells of the nervous system maintain their energy balance through the use of ketone bodies (62). We

propose that CD8^{+} TILs may do the same. T cells may take up ketone bodies from the surrounding or they could synthesize them directly (61, 63, 64).

CD8^{+} TILs are not stationary; they migrate throughout the TME (65) and we assume that their environment changes accordingly. When T cells are close to vessels and O_2 is readily available, FAs may fuel the TCA cycle and excess FA-derived acetyl-CoA can be converted into ketone bodies. When T cells penetrate deeply into the tumor and O_2 becomes scarce, T cells may burn ketone bodies to sustain their energy requirement. As has been shown during heart ischemia, lack of O_2 results in increases in AMPK, which decreases the activity of acetyl-CoA carboxylase (ACC) that converts acetyl-CoA to malonyl-CoA. Once cells have access to O_2, such upon reperfusion of an ischemic heart or migration of TILs to areas close to vasculature, lack of malonyl-CoA will cause a surge in FAO (66). Lipid metabolism has been shown to correlate with long-term survival in many different cell types (67, 68). It has been suggested that PD-1 ligation, which prevents terminal differentiation of effector CD8^{+} T cells (69), promotes survival by enhancing the cells' FA metabolism (10). Ketone body metabolism also promotes metabolic fitness and longevity of cells by regulating histone deacetylase (HDAC) activities (61). Catabolism of these carbon sources may allow T cells to survive under hypoglycemia and intermittent hypoxia.

EXPLORING METABOLIC MANIPULATIONS TO IMPROVE T CELL-MEDIATED IMMUNOTHERAPY OF CANCER

Adoptive transfer of *ex vivo* expanded TILs has achieved some successes in treatment of melanomas (70). Alternatively, T cells from peripheral blood can be modified to express chimeric antigen receptors (CARs) that recognize cell surface expressed TAs independent of major histocompatibility antigens (71). Transfer of such CAR-T cells has been remarkably successful in treatment of acute lymphatic leukemia or B cell lymphoma but in general yielded disappointing results in patients with solid tumors (72–76). This has been blamed on the immunosuppressive nature of the TME (77, 78). Treatments that reduce numbers or functions of regulatory T cells, myeloid suppressor cells, tumor-associated macrophages, or that block immune checkpoints have improved the efficacy of adoptive cell transfer for cancer therapy (79, 80).

As already mentioned, the metabolic profile of CD8^{+} T cells prior to their tumor infiltration has significant impacts on their longevity and performance within TME. In addition, the metabolic profiles of the tumor cells will influence what nutrients are available to T cells. Some studies show that drugs, which inhibit glycolysis by tumor cells such as Glut1 inhibitors, cause tumor regression and increase glucose supply within the TME (81, 82). Its use prior to cell transfer could increase the efficacy of cell immunotherapy. Others have shown that reducing glucose consumption by tumor cells through blockade of PD-L1 signaling allows for increased glycolytic energy production by CD8^{+} TILs, which is accompanied by improvements of their functions (39).

A recent study shows that glucose limitation leads to reduction of the glycolysis metabolite phosphoenoylpruvate (PEP), which is essential for Ca^{2+}-NFAT signaling in CD8^{+} TILs. Overexpressing phosphoenolpyruvate carboxykinase (PCK) 1, which converts the TCA cycle intermediate oxaloacetate to PEP, was shown to improve NFAT signaling and function of TILs (44).

However, glycolysis may accelerate terminal differentiation of CD8^{+} T cells, and thereby shorten T cell survival (83); thereby, limiting glycolysis by CD8^{+} TILs may yield better therapeutic effects. One study showed that inhibition of glycolysis by 2-deoxyglucose during *in vitro* expansion of TA-specific CD8^{+} T cells increases their efficacy in a mouse melanoma model (52). A similar effect was seen when TILs were treated prior to adoptive transfer with an Akt inhibitor, which reduces their use of glycolysis and increases OXPHOS (57). Along the same line, when CD8^{+} T cells were cultured *in vitro* with IL-7 or IL-15, which drives their differentiation toward memory, their antitumor efficacies *in vivo* significantly improved (84–86). Further studies showed that adoptive transfer of central memory CD8^{+} T cells confers better eradication of solid tumor masses than transfer of equal numbers of effector CD8^{+} T cells (87, 88), which led the authors to conclude that the T cell differentiation status, which dictates potential for proliferation, is crucial to ensure optimal efficacy of adoptive T cell transfer. We think that the metabolic reprograming toward preferential use of FAO and OXPHOS as naturally occurs during differentiation from effector to memory cells, may allow for the superior performance of the transferred T cells within TME.

CONCLUSION

Immunotherapy of cancer is still in its infancy. T cells are able to stop an infection by rapidly killing millions of infected cells but they clearly need additional help to eliminate tumors. In recent years, exciting new studies have started to illuminate the metabolism of T cells under different conditions and its impact on T differentiation and functions. A better understanding of the metabolic programs utilized by CD8^{+} TILs and how they affect the TILs' antitumor performance is crucial to find new therapeutic targets for cancer immunotherapy. Metabolic manipulations that could prepare TA-specific T cells, which are either induced by cancer vaccines or expanded *ex vivo* in form of TILs or CAR-T cells, to optimally cope with the metabolic constrains within the TME may significantly improve the overall antitumor efficacy. Undoubtedly, the type of cancer and peculiarities of its TME will dictate the most suited metabolic treatment.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. *Nat Rev Immunol* (2005) **5**:844–52. doi:10.1038/nri1710

2. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* (2002) **16**:769–77. doi:10.1016/S1074-7613(02)00323-0

3. Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, et al. Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res* (2004) **64**:3892–9. doi:10.1158/0008-5472.CAN-03-2904

4. Dang CV. MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med* (2013) **3**:a014217–014217. doi:10.1101/cshperspect.a014217

5. Antico Arciuch VG, Russo MA, Kang KS, Di Cristofano A. Inhibition of AMPK and Krebs cycle gene expression drives metabolic remodeling of Pten-deficient preneoplastic thyroid cells. *Cancer Res* (2013) **73**:5459–72. doi:10.1158/0008-5472.CAN-13-1429

6. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol* (2012) **13**:907–15. doi:10.1038/ni.2386

7. Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol* (2015) **36**:81–91. doi:10.1016/j.it.2014.12.005

8. O'Sullivan D, van der Windt GJW, Huang SC-C, Curtis JD, Chang C-H, Buck MD, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity* (2014) **41**:75–88. doi:10.1016/j.immuni.2014.06.005

9. Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol* (2005) **25**:9543–53. doi:10.1128/MCB.25.21.9543-9553.2005

10. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun* (2015) **6**:6692. doi:10.1038/ncomms7692

11. Charlton JJ, Chatzidakis I, Tsoukatou D, Boumpas DT, Garinis GA, Mamalaki C. Programmed death-1 shapes memory phenotype CD8 T cell subsets in a cell-intrinsic manner. *J Immunol* (2013) **190**:6104–14. doi:10.4049/jimmunol.1201617

12. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* (2006) **439**:682–7. doi:10.1038/nature04444

13. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* (2009) **10**:29–37. doi:10.1038/ni.1679

14. Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol* (2013) **25**:214–21. doi:10.1016/j.coi.2012.12.003

15. Mahoney KM, Rennert PD, Freeman GJ. Combination cancer immunotherapy and new immunomodulatory targets. *Nat Rev Drug Discov* (2015) **14**:561–84. doi:10.1038/nrd4591

16. Ruiz-Cabello F, Nevot MAL, Garrido F. MHC class I and II gene expression on human tumors. *Adv Exp Med Biol* (1988) **233**:119–28. doi:10.1007/978-1-4899-5037-6_14

17. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev* (2007) **26**:225–39. doi:10.1007/s10555-007-9055-1

18. Firth JD, Ebert BL, Pugh CW, Ratcliffe PJ. Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: similarities with the erythropoietin 3' enhancer. *Proc Natl Acad Sci U S A* (1994) **91**:6496–500. doi:10.1073/pnas.91.14.6496

19. Kim J-W, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* (2006) **3**:177–85. doi:10.1016/j.cmet.2006.02.002

20. Sitkovsky MV, Lukashev D, Apasov S, Kojima H, Koshiba M, Caldwell C, et al. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. *Annu Rev Immunol* (2004) **22**:657–82. doi:10.1146/annurev.immunol.22.012703.104731

21. Atkuri KR, Herzenberg LA, Herzenberg LA. Culturing at atmospheric oxygen levels impacts lymphocyte function. *Proc Natl Acad Sci U S A* (2005) **102**:3756–9. doi:10.1073/pnas.0409910102

22. Larbi A, Zelba H, Goldeck D, Pawelec G. Induction of HIF-1alpha and the glycolytic pathway alters apoptotic and differentiation profiles of activated human T cells. *J Leukoc Biol* (2010) **87**:265–73. doi:10.1189/jlb.0509304

23. Zuckerberg AL, Goldberg LI, Lederman HM. Effects of hypoxia on interleukin-2 mRNA expression by T lymphocytes. *Crit Care Med* (1994) **22**:197–203. doi:10.1097/00003246-199402000-00008

24. Kim H, Peng G, Hicks JM, Weiss HL, Van Meir EG, Brenner MK, et al. Engineering human tumor-specific cytotoxic T cells to function in a hypoxic environment. *Mol Ther* (2008) **16**:599–606. doi:10.1038/sj.mt.6300391

25. Neumann AK, Yang J, Biju MP, Joseph SK, Johnson RS, Haase VH, et al. Hypoxia inducible factor 1 alpha regulates T cell receptor signal transduction. *Proc Natl Acad Sci U S A* (2005) **102**:17071–6. doi:10.1073/pnas.0506070102

26. Choukèr A, Thiel M, Lukashev D, Ward JM, Kaufmann I, Apasov S, et al. Critical role of hypoxia and A2A adenosine receptors in liver tissue-protecting physiological anti-inflammatory pathway. *Mol Med* (2008) **14**:116–23. doi:10.2119/2007-00075.Chouker

27. Meehan R, Duncan U, Neale L, Taylor G, Muchmore H, Scott N, et al. Operation Everest II: alterations in the immune system at high altitudes. *J Clin Immunol* (1988) **8**:397–406. doi:10.1007/BF00917156

28. Bell EL, Klimova TA, Eisenbart J, Moraes CT, Murphy MP, Budinger GRS, et al. The Qo site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production. *J Cell Biol* (2007) **177**:1029–36. doi:10.1083/jcb.200609074

29. Kesarwani P, Murali AK, Al-Khami AA, Mehrotra S. Redox regulation of T-cell function: from molecular mechanisms to significance in human health and disease. *Antioxid Redox Signal* (2013) **18**:1497–534. doi:10.1089/ars.2011.4073

30. Guo J, Lu W, Shimoda LA, Semenza GL, Georas SN. Enhanced interferon-gamma gene expression in T cells and reduced ovalbumin-dependent lung eosinophilia in hypoxia-inducible factor-1-alpha-deficient mice. *Int Arch Allergy Immunol* (2009) **149**:98–102. doi:10.1159/000189191

31. Lukashev D, Klebanov B, Kojima H, Grinberg A, Berenfeld L, Wenger RH, et al. Cutting edge: hypoxia-inducible factor 1 and its activation-inducible short isoform I.1 negatively regulate functions of CD4+ and CD8+ T lymphocytes. *J Immunol* (2006) **177**:4962–5. doi:10.4049/jimmunol.177.8.4962

32. Doedens AL, Phan AT, Stradner MH, Fujimoto JK, Nguyen JV, Yang E, et al. Hypoxia-inducible factors enhance the effector responses of CD8(+) T cells to persistent antigen. *Nat Immunol* (2013) **14**:1173–82. doi:10.1038/ni.2714

33. Finlay DK, Rosenzweig E, Sinclair LV, Feijoo-Carnero C, Hukelmann JL, Rolf J, et al. PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. *J Exp Med* (2012) **209**:2441–53. doi:10.1084/jem.20112607

34. Bos R, van der Groep P, Greijer AE, Shvarts A, Meijer S, Pinedo HM, et al. Levels of hypoxia-inducible factor-1 alpha independently predict prognosis in patients with lymph node negative breast carcinoma. *Cancer* (2003) **97**:1573–81. doi:10.1002/cncr.11246

35. Swinson DEB, Jones JL, Cox G, Richardson D, Harris AL, O'Byrne KJ. Hypoxia-inducible factor-1 alpha in non small cell lung cancer: relation to growth factor, protease and apoptosis pathways. *Int J Cancer* (2004) **111**:43–50. doi:10.1002/ijc.20052

36. Barsoum IB, Smallwood CA, Siemens DR, Graham CH. A mechanism of hypoxia-mediated escape from adaptive immunity in cancer cells. *Cancer Res* (2014) **74**:665–74. doi:10.1158/0008-5472.CAN-13-0992

37. Fang H-Y, Hughes R, Murdoch C, Coffelt SB, Biswas SK, Harris AL, et al. Hypoxia-inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. *Blood* (2009) **114**:844–59. doi:10.1182/blood-2008-12-195941

38. Nizet V, Johnson RS. Interdependence of hypoxic and innate immune responses. *Nat Rev Immunol* (2009) **9**:609–17. doi:10.1038/nri2607

39. Chang C-H, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* (2015) **162**:1–14. doi:10.1016/j.cell.2015.08.016

40. Cham CM, Gajewski TF. Glucose availability regulates IFN-gamma production and p70S6 kinase activation in CD8+ effector T cells. *J Immunol* (2005) **174**:4670–7. doi:10.4049/jimmunol.174.8.4670

41. Cham CM, Driessens G, O'Keefe JP, Gajewski TF. Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+ T cells. *Eur J Immunol* (2008) 38:2438–50. doi:10.1002/eji.200838289

42. Jacobs SR, Herman CE, Maciver NJ, Wofford JA, Wieman HL, Hammen JJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol* (2008) 180:4476–86. doi:10.4049/jimmunol.180.7.4476

43. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* (2014) 20:61–72. doi:10.1016/j.cmet.2014.05.004

44. Ho P-C, Bihuniak JD, Macintyre AN, Staron M, Liu X, Amezquita R, et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* (2015) 162:1217–28. doi:10.1016/j.cell.2015.08.012

45. Warburg O. On the origin of cancer cells. *Science* (1956) 123:309–14. doi:10.1126/science.123.3191.309

46. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, et al. Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood* (2007) 109:3812–9. doi:10.1182/blood-2006-07-035972

47. Chang C-H, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* (2013) 153:1239–51. doi:10.1016/j.cell.2013.05.016

48. Buzzai M, Bauer DE, Jones RG, DeBerardinis RJ, Hatzivassiliou G, Elstrom RL, et al. The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid β-oxidation. *Oncogene* (2005) 24:4165–73. doi:10.1038/sj.onc.1208622

49. Blagih J, Coulombe F, Vincent EE, Dupuy F, Galicia-Vázquez G, Yurchenko E, et al. The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. *Immunity* (2015) 42:41–54. doi:10.1016/j.immuni.2014.12.030

50. O'Neill LAJ, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature* (2013) 493:346–55. doi:10.1038/nature11862

51. O'Sullivan D, Pearce EL. Targeting T cell metabolism for therapy. *Trends Immunol* (2015) 36:71–80. doi:10.1016/j.it.2014.12.004

52. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest* (2013) 123:4479–88. doi:10.1172/JCI69589

53. Manjunath N, Shankar P, Wan J, Weninger W, Crowley MA, Hieshima K, et al. Effector differentiation is not prerequisite for generation of memory cytotoxic T lymphocytes. *J Clin Invest* (2001) 108:871–8. doi:10.1172/JCI13296

54. Maciver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL, Rathmell JC. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J Leukoc Biol* (2008) 84:949–57. doi:10.1189/jlb.0108024

55. Crompton JG, Sukumar M, Roychoudhuri R, Clever D, Gros A, Eil RL, et al. Akt inhibition enhances expansion of potent tumor-specific lymphocytes with memory cell characteristics. *Cancer Res* (2015) 75:296–305. doi:10.1158/0008-5472.CAN-14-2277

56. Currie E, Schulze A, Zechner R, Walther TC, Farese RV. Cellular fatty acid metabolism and cancer. *Cell Metab* (2013) 18:153–61. doi:10.1016/j.cmet.2013.05.017

57. Zhang F. Dysregulated lipid metabolism in cancer. *World J Biol Chem* (2012) 3:167. doi:10.4331/wjbc.v3.i8.167

58. Zhang Y, Bellows CF, Kolonin MG. Adipose tissue-derived progenitor cells and cancer. *World J Stem Cells* (2010) 2:103–13. doi:10.4252/wjsc.v2.i5.103

59. Byersdorfer CA, Tkachev V, Opipari AW, Goodell S, Swanson J, Sandquist S, et al. Effector T cells require fatty acid metabolism during murine graft-versus-host disease. *Blood* (2013) 122:3230–7. doi:10.1182/blood-2013-04-495515

60. Byersdorfer CA. The role of fatty acid oxidation in the metabolic repro-graming of activated T-cells. *Front Immunol* (2014) 5:161. doi:10.3389/fimmu.2014.00641

61. Newman JC, Verdin E. Ketone bodies as signaling metabolites. *Trends Endocrinol Metab* (2014) 25:42–52. doi:10.1016/j.tem.2013.09.002

62. Takahashi S, Iizumi T, Mashima K, Abe T, Suzuki N. Roles and regulation of ketogenesis in cultured astroglia and neurons under hypoxia and hypoglyce-mia. *ASNNeuro* (2014) 6:1759091414550997. doi:10.1177/1759091414550997

63. Curi R, Williams JF, Newsholme EA. Formation of ketone bodies by resting lymphocytes. *Int J Biochem* (1989) 21:1133–6. doi:10.1016/0020-711X(89)90055-4

64. Curi R, Williams JF, Newsholme EA. Pyruvate metabolism by lymphocytes: evidence for an additional ketogenic tissue. *Biochem Int* (1989) 19:755–67.

65. Mrass P, Takano H, Ng LG, Daxini S, Lasaro MO, Iparraguirre A, et al. Random migration precedes stable target cell interactions of tumor-infiltrating T cells. *J Exp Med* (2006) 203:2749–61. doi:10.1084/jem.20060710

66. Kudo N, Barr AJ, Barr RL, Desai S, Lopaschuk GD. High rates of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA levels due to an increase in 5'-AMP-activated protein kinase inhibition of acetyl-CoA carboxylase. *J Biol Chem* (1995) 270:17513–20. doi:10.1074/jbc.270.29.17513

67. van der Windt GJW, Pearce EL. Metabolic switching and fuel choice during T-cell differentiation and memory development. *Immunol Rev* (2012) 249:27–42. doi:10.1111/j.1600-065X.2012.01150.x

68. Zaugg K, Yao Y, Reilly PT, Kannan K, Kiarash R, Mason J, et al. Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. *Genes Dev* (2011) 25:1041–51. doi:10.1101/gad.1987211

69. Odorizzi PM, Pauken KE, Paley MA, Sharpe A, Wherry EJ. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8+ T cells. *J Exp Med* (2015) 212:1125–37. doi:10.1084/jem.20142237

70. Hinrichs CS, Rosenberg SA. Exploiting the curative potential of adoptive T-cell therapy for cancer. *Immunol Rev* (2014) 257:56–71. doi:10.1111/imr.12132

71. Maus MV, Powell DJ. Chimeric antigen receptor T-cells: new approaches to improve their efficacy and reduce toxicity. *Cancer J* (2015) 21:475–9. doi:10.1097/PPO.0000000000000155

72. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemo-therapy-refractory acute lymphoblastic leukemia. *Sci Transl Med* (2013) 5:177ra38. doi:10.1126/scitranslmed.3005930

73. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* (2013) 368:1509–18. doi:10.1056/NEJMoa1215134

74. Kakarla S, Gottschalk S. CAR T cells for solid tumors. *Cancer J* (2014) 20:151–5. doi:10.1097/PPO.0000000000000032

75. Park JR, Digiusto DL, Slovak M, Wright C, Naranjo A, Wagner J, et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. *Mol Ther* (2007) 15:825–33. doi:10.1038/sj.mt.6300104

76. Lamers CHJ. Treatment of metastatic renal cell carcinoma with autol-ogous t-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol* (2006) 24:e20–2. doi:10.1200/JCO.2006.05.9964

77. Curran KJ, Brentjens RJ. Chimeric antigen receptor T cells for cancer immu-notherapy. *J Clin Oncol* (2015) 33:1703–6. doi:10.1200/JCO.2014.60.3449

78. Beatty GL, Moon EK. Chimeric antigen receptor T cells are vulnerable to immunosuppressive mechanisms present within the tumor microenviron-ment. *OncoImmunology* (2014) 3:e970027. doi:10.4161/21624011.2014.970027

79. Beavis PA, Slaney CY, Kershaw MH, Gyorki D, Neeson PJ, Darcy PK. Reprogramming the tumor microenvironment to enhance adoptive cellular therapy. *Semin Immunol* (2015). doi:10.1016/j.smim.2015.11.003

80. John LB, Kershaw MH, Darcy PK. Blockade of PD-1 immunosuppression boosts CAR T-cell therapy. *OncoImmunology* (2013) 2:e26286. doi:10.4161/onci.26286

81. Liu Y, Cao Y, Zhang W, Bergmeier S, Qian Y, Akbar H, et al. A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth in vitro and in vivo. *Mol Cancer Ther* (2012) 11:1672–82. doi:10.1158/1535-7163.MCT-12-0131

82. Birsoy K, Wang T, Possemato R, Yilmaz OH, Koch CE, Chen WW, et al. MCT1-mediated transport of a toxic molecule is an effective strategy for targeting glycolytic tumors. *Nat Genet* (2013) 45:104–8. doi:10.1038/ng.2471

83. Gattinoni L, Klebanoff CA, Palmer DC, Wrzesinski C, Kerstann K, Yu Z, et al. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8+ T cells. *J Clin Invest* (2005) 115:1616–26. doi:10.1172/JCI24480

84. Zeng R, Spolski R, Finkelstein SE, Oh S, Kovanen PE, Hinrichs CS, et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. *J Exp Med* (2005) 201:139–48. doi:10.1084/jem.20041057

85. Liu S, Lizée G, Lou Y, Liu C, Overwijk WW, Wang G, et al. IL-21 synergizes with IL-7 to augment expansion and anti-tumor function of cytotoxic T cells. *Int Immunol* (2007) **19**:1213–21. doi:10.1093/intimm/dxm093

86. Fewkes NM, Mackall CL. Novel gamma-chain cytokines as candidate immune modulators in immune therapies for cancer. *Cancer J* (2010) **16**:392–8. doi:10.1097/PPO.0b013e3181eacbc4

87. Klebanoff CA, Gattinoni L, Torabi-Parizi P, Kerstann K, Cardones AR, Finkelstein SE, et al. Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. *Proc Natl Acad Sci U S A* (2005) **102**:9571–6. doi:10.1073/pnas.0503726102

88. Klebanoff CA, Gattinoni L, Palmer DC, Muranski P, Ji Y, Hinrichs CS, et al. Determinants of successful CD8+ T-cell adoptive immunotherapy for large established tumors in mice. *Clin Cancer Res* (2011) **17**:5343–52. doi:10.1158/1078-0432.CCR-11-0503

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