Nutrient Competition: A New Axis of Tumor Immunosuppression

Madhusudhanan Sukumar,[1] Rahul Roychoudhuri,[1] and Nicholas P. Restifo[1,*]
[1]Center for Cancer Research, National Cancer Institute (NCI), National Institutes of Health Bethesda, MD 20892, USA
*Correspondence: restifo@nih.gov
http://dx.doi.org/10.1016/j.cell.2015.08.064

It is thought that cancer cells engage in Warburg metabolism to meet intrinsic biosynthetic requirements of cell growth and proliferation. Papers by Chang et al. and Ho et al. show that Warburg metabolism enables tumor cells to restrict glucose availability to T cells, suppressing anti-tumor immunity.

In the presence of oxygen, most differentiated cells utilize mitochondrial oxidative phosphorylation to generate energy in the form of adenosine triphosphate (ATP) that can be used to sustain cellular processes. In the absence of oxygen, such cells revert to much less efficient glycolysis as a means of ATP production. Cancer cells often utilize glycolysis despite the presence of oxygen (aerobic glycolysis or the "Warburg effect") (Warburg, 1956). While less efficient at producing energy, it is thought that this form of metabolism supports the macromolecular requirements of cell growth and proliferation. Thus, the field has primarily focused on Warburg metabolism as an adaptation that confers intrinsic growth advantages to tumor cells themselves. However, cancer cells may consume nutrients, particularly glucose, in excess of their requirement to sustain proliferation and cell growth (Vander Heiden et al., 2009). This raises the possibility that nutrient consumption serves additional roles to meeting the intrinsic bioenergetic and biosynthetic requirements of cancer cells. In this issue of *Cell*, Ho et al. (2015) and Chang et al. (2015) show that Warburg metabolism provides tumor cells with a cell-extrinsic advantage, promoting depletion of extracellular glucose which renders tumor-infiltrating T cells dysfunctional.

In both studies, glycolysis within tumor cells is shown to cause depletion of extracellular glucose which restricts glucose availability to T cells. Decreased glucose availability causes suppression of glycolytic metabolism within T cells, and this is associated with decreased effector function (Figure 1, left). Ho et al. identify a mechanism by which glucose metabolism directly controls effector function. The authors find that T cell receptor (TCR)-induced Ca^{2+} flux is markedly dependent upon extracellular glucose and glucose metabolism by T cells. Sarco/endoplasmic reticulum (ER) Ca^{2+}-ATPase (SERCA) is an ATP-dependent Ca^{2+} channel that pumps Ca^{2+} from the cytoplasm into the ER. Extracellular glucose is shown to promote accumulation of the glycolytic metabolite, phosphoenolpyruvate (PEP), which inhibits SERCA-dependent evacuation of Ca^{2+} from the cytosol into the ER, thereby increasing TCR-induced Ca^{2+} flux and effector function (Figure 1, right). This observation adds to a growing list of examples whereby metabolic processes directly control the outcome of T cell activation (Chang et al., 2013; MacIver et al., 2013).

That tumor cell glycolysis directly suppresses T cells raises the possibility that tumor metabolism can be therapeutically manipulated to improve immune function within tumors. Checkpoint blockade immunotherapy with anti-PD-L1 antibodies is thought to work by limiting inhibitory PD-1 signaling received by tumor-specific T cells (Keir et al., 2008). Chang et al. made the surprising observation that PD-1 ligand (PD-L1) expressed by tumor cells provides a constitutive "reverse signal" that promotes tumor cell glycolysis through activation of the AKT/mTOR pathway (Figure 1, left). Treatment of tumor cells with therapeutic anti-PD-L1 antibodies attenuates glycolysis by triggering PD-L1 endocytosis (Figure 1, right). Remarkably, two other checkpoint-blockade antibodies, anti-PD-1 and anti-CTLA-4, are also shown to cause changes in extracellular glucose concentrations within tumors, though mechanisms for these observations are unclear. That PD-L1 expression causes constitutive activation of the Akt/mTOR pathway has important implications for understanding tumor cell biology and tumor-host interactions, and it will be important to characterize precise molecular mechanisms by which PD-L1 constitutively activates the Akt/mTOR pathway. Given that immune checkpoint blockade elicits durable clinical responses and improves survival in patients with certain metastatic cancers (Larkin et al., 2015; Topalian et al., 2012), it is relevant to measure the effect of checkpoint blockade antibodies on intratumoral nutrient availability and T cell metabolism in patients and correlate this with clinical outcomes. Further, it will be important to dissect the effects of checkpoint blockade on inhibitory T cell signaling versus tumor cell metabolism.

Instead of manipulating tumor cell metabolism, Ho et.al. suggest an alternate approach to improve T cell function by mimicking nutrient availability within transferred T cells during adoptive cell therapy (ACT). Phosphoenolpyruvate Carboxykinase (PCK1) converts oxaloacetate into PEP. By overexpressing Pck1 in transferred T cells, Ho et al. are able to artificially increase PEP levels, restoring TCR-induced Ca^{2+} flux and anti-tumor T cell function despite the presence of low environmental glucose levels within tumors. Intriguingly, blocking glucose

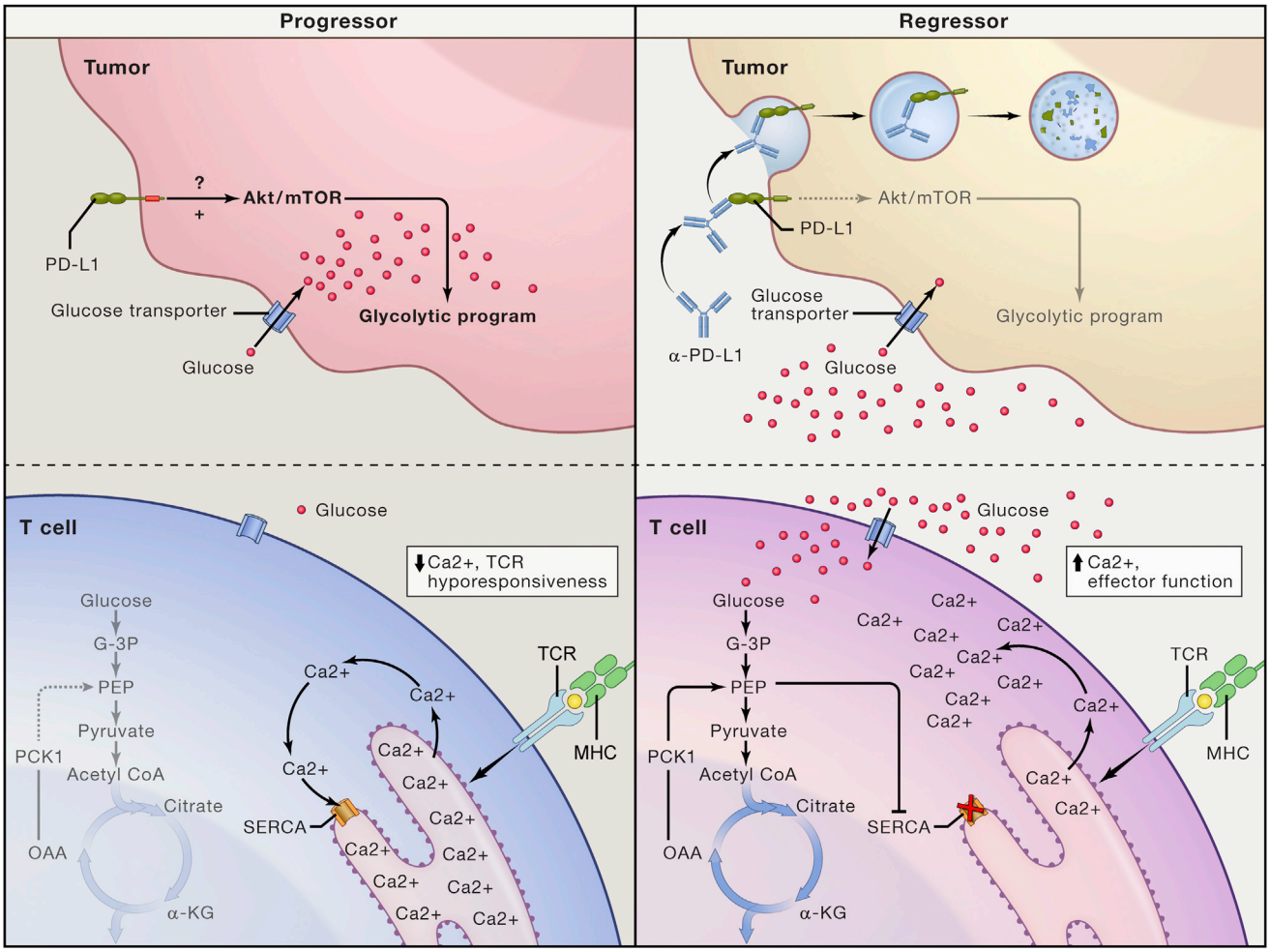

Figure 1. Nutrient Competition between Tumor Cells and T Cells Controls Immune Function within Tumors

Schematic depicting glucose metabolism and cellular signaling in highly glycolytic progressor tumors and repressor tumors undergoing therapy. In the progressor tumor (left), constitutive activation of the Akt/mTOR pathway by PD-L1 expressed on tumor cells causes high levels of tumor cell glycolysis and absorption of extracellular glucose. Decreased extracellular glucose levels causes impaired glycolysis in T cells, wherein depletion of the glycolytic metabolite PEP causes unrestrained SERCA activity, sequestration of cytoplasmic $Ca2^+$ into the ER and impairment of TCR-induced $Ca2^+$ flux and effector function. In the regressor tumor (right), therapeutic anti-PD-L1 antibodies bind to PD-L1 causing its endocytosis and inactivation. Loss of constitutive PD-L1 signaling leads to decreased activation of the Akt-mTOR pathway decreased tumor cell glycolysis and increased extracellular glucose concentrations. Increased extracellular glucose drives T cell glycolysis, replenishing PEP levels, inhibiting SERCA-dependent sequestration of cytoplasmic $Ca2^+$ and promoting TCR-induced $Ca2^+$ flux and anti-tumor effector functions. Alternatively, constitutive overexpression of PCK1 in adoptively transferred T cells increases availability of PEP leading to inhibition of SERCA, increased anti-tumor effector function and tumor regression.

metabolism during expansion of T cells for adoptive immunotherapy withholds effector differentiation and promotes differentiation of memory cells which mediate superior tumor clearance (Sukumar et al., 2013). These findings provide striking examples of how modulating T cell metabolism can improve the outcome of adoptive cell therapy for cancer.

Taken together, the two new studies provide compelling evidence that cancer cells subvert the metabolic characteristics of the tumor microenvironment to shape immune responses within tumors. The results also provide an explanation of how nutrient consumption in excess of the bioenergetic and biosynthetic requirements may benefit cancer cells. As Warburg's original observation is revisited in ever new reincarnations, it remains to be seen whether insights from the field of immunometabolism will change the game at this new front in our war against cancer.

REFERENCES

Chang, C.H., Curtis, J.D., Maggi, L.B., Jr., Faubert, B., Villarino, A.V., O'Sullivan, D., Huang, S.C., van der Windt, G.J., Blagih, J., Qiu, J., et al. (2013). Cell *153*, 1239–1251.

Chang, C.-H., Qiu, J., O'Sullivan, D., Buck, M.D., Noguchi, T., Curtis, J.D., Chen, Q., Gindin, M., Gubin, M.W., and van der Windt, G.J.W. (2015). Cell *162*, this issue, 1229–1241.

Ho, P.-C., Bihuniak, J.D., Macintyre, A.N., Staron, M., Liu, X., Amezquita, R., Tsui, Y.-C., Cui, G., Micevic, G., Perales, J.C., et al. (2015). Cell *162*, this issue, 1217–1228.

Keir, M.E., Butte, M.J., Freeman, G.J., and Sharpe, A.H. (2008). Annu. Rev. Immunol. *26*, 677–704.

Larkin, J., Chiarion-Sileni, V., Gonzalez, R., Grob, J.J., Cowey, C.L., Lao, C.D., Schadendorf, D., Dummer, R., Smylie, M., Rutkowski, P., et al. (2015). N. Engl. J. Med. *373*, 23–34.

MacIver, N.J., Michalek, R.D., and Rathmell, J.C. (2013). Annu. Rev. Immunol. *31*, 259–283.

Sukumar, M., Liu, J., Ji, Y., Subramanian, M., Crompton, J.G., Yu, Z., Roychoudhuri, R., Palmer, D.C., Muranski, P., Karoly, E.D., et al. (2013). J. Clin. Invest. *123*, 4479–4488.

Topalian, S.L., Hodi, F.S., Brahmer, J.R., Gettinger, S.N., Smith, D.C., McDermott, D.F., Powderly, J.D., Carvajal, R.D., Sosman, J.A., Atkins, M.B., et al. (2012). N. Engl. J. Med. *366*, 2443–2454.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Science *324*, 1029–1033.

Warburg, O. (1956). Science *123*, 309–314.

Single-Cell Analysis: The Differences That Kill

Savaş Tay[1,2,*]
[1]Department of Biosystems Science and Engineering, ETH Zurich, Basel 4058, Switzerland
[2]Institute for Molecular Engineering, University of Chicago, Chicago 60637, USA
*Correspondence: tays@uchicago.edu
http://dx.doi.org/10.1016/j.cell.2015.08.053

Using single-cell RNA sequencing, Avraham et al. investigate how variability in macrophage response to infection is controlled by variability within the pathogen population. They find that heterogeneous expression of the *Salmonella* virulence factor PhoP and subsequent cell-wall modifications lead to the bimodal induction of the interferon-response in infected macrophages.

What exactly happens when pathogens penetrate the outer defenses of tissues and start infecting various cells? Since the dawn of modern biology, the battle between pathogens and immune cells has been a central focus, and thanks to powerful new methods that analyze individual cells, we are taking a fresh look at our understanding of infection and immunity. Unlike what traditional population-averaged analyses show, the outcome of pathogen exposure is vastly more complex at the individual-cell level. For example, some host cells completely avoid infection and survive. Other cells become infected and die, survive with the presence of bacteria inside them, or completely clear the pathogens and function normally afterward. The intricate workings of the molecular pathways determining infection and immunity are largely unclear. In this issue of *Cell*, Hung and colleagues take a new look at this fundamental problem using single-cell analysis and ask whether variability in infection outcomes can be explained by the variability among individual bacteria (Avraham et al., 2015). This is a unique approach as compared to most work in the newly emerging field of single-cell immunology. In explaining heterogeneous infection outcomes, the field tends to focus on the state of the host and environment (Snijder et al., 2009), rather than pre-existing variability in the pathogen.

Hung's team focus on the infection of macrophages—first responders of the innate immune system—with *Salmonella typhimurium*, a pathogen that causes typhoid fever and food poisoning in humans. Despite a century of antibiotic treatment and improved hygiene, basic pathogens such as *Salmonella* remain a major health problem, especially in the developing world. Even the developed world is at risk from these basic infections, as evidenced by thousands of *salmonella* infections every year in the USA alone and the recent *E. coli* outbreak in Germany that killed 50 people over the course of a few weeks.

Salmonella typhimurium has specialized molecular tools to avoid, resist, and even hijack the mammalian immune system. Macrophages recognize these pathogen-associated factors and mount transcriptional programs to change their physiology and clear the pathogen. Individual *Salmonella* cells can vary in the manner they express virulence factors. Can the variability in infection outcomes be explained by the variability within the pathogen population? And if so, what virulence factors control this variability? To answer these questions, Avraham et al. first use fluorescent single-cell microscopy to distinguish various infection outcomes: When mixed with *salmonella*, the macrophages could remain uninfected, or become infected with either live or dead bacteria inside. They isolate these single macrophages and use state-of-the-art RNA sequencing (RNA-seq) to determine their transcriptional state by measuring the expression of 535 immune response genes. These genes cluster into distinct groups; however, one cluster shows much higher expression variability between individual cells. These variable genes were related to innate immune recognition of the bacterial virulence factors, including bacterial cell-wall components like lipopolysaccharide (LPS), hinting that the LPS/TLR4 signaling pathway underlies phenotype variability. In particular, Type 1 interferon (IFN) response exhibit bimodal expression in host macrophages, with roughly one third of cells expressing IFN genes at high levels, and the rest at low levels