Author/s:
Gubser, PM;Kallies, A

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Strapline: Epigenetic modification by methionine starvation

Methio-“mine”! Cancer cells steal methionine and impair CD8 T cell function

Patrick M Gubser and Axel Kallies

Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, 792 Elizabeth St, Melbourne VIC 3000, Australia.

axel.kallies@unimelb.edu.au

Cytotoxic CD8 T cells can eradicate cells that are pathogen-infected or have undergone malignant transformation. However, tumor infiltrating CD8 T cells undergo functional and phenotypic changes, leading to increased expression of inhibitory receptors or checkpoints, such as PD-1, and impaired effector cytokine production, a state termed ‘exhaustion’. These functional changes are also reflected by epigenetic modifications at gene loci encoding effector molecules, including interferon γ (IFNγ) and granzymes (1). Checkpoint inhibition, such as PD-1/PD-L1

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blockade, can activate exhausted CD8 T cells and has revolutionised cancer therapy in the past decade. However, the epigenetic stability of exhausted T cells limits the magnitude and durability of the reinvigoration the T cell response by checkpoint inhibition and thus constitutes a substantial hurdle to further improve immunotherapy (2). In a recent study published in *Nature*, Bian *et al.* show that cancer cell metabolism mediated the depletion of the essential amino acid methionine from the tumor microenvironment (TME) and show that this can directly impact the epigenetic landscape of infiltrating CD8 T cells and impair their anti-tumor function (3).

Nearly a century ago, Otto Warburg established that cancer cells depend on glycolysis and convert pyruvate into lactate even in the presence of oxygen (4). Although less efficient than oxidation, this process of aerobic glycolysis enables tumor cells to fuel their rapid anabolic growth and proliferation by shuttling intermediates into various biosynthetic pathways (5). However, glucose is not the only energy source for cancer cells. Indeed, high uptake of amino acids, especially glutamine, is another hallmark of cancer cell metabolism. As a consequence, not only glucose but also amino acids are depleted from the TME while waste products accumulate (6). Activated T cells share many metabolic similarities with cancer cells. Activation induced increased glucose and amino acid uptake is crucial for T cell proliferation and acquisition of effector function (7). Bian *et al.* now provide another intriguing example for the competition between cancer and immune cells and how this process impairs tumor infiltrating CD8 T cells (3).

Bian *et al.* show that culturing CD8 T cells in supernatants of various cancer cell lines induced apoptosis in the T cells and impaired their effector function unless the supernatant was supplemented with methionine (3). Using a transwell system with varying methionine concentrations, the authors further showed that tumor cells outcompeted T cells for methionine. Although methionine can also be generated *de novo* in mammalian cells, recent studies have shown that T cells are dependent on methionine uptake via the system L transporter *Solute Carrier Family 7 Member 5* (SLC7A5) (8, 9). Unlike T cells, which only express SLC7A5, tumor cells in addition express high amounts of a second system L transporter, namely SLC43A2. By knocking down SLC43A2 in a mouse melanoma cell line, Bian *et al.* could confirm that this additional transporter enables tumor cells to outcompete T cells for

methionine uptake (**Figure 1**). Thus, pharmacological inhibition of SLC43A2 may be a promising approach for a cancer therapy. Indeed, in a series of different human cancers, Bian *et al.* could correlate high tumor expression of SLC43A2 with poor patient survival. Unfortunately, a specific SLC43A2 inhibitor is not available currently and inhibiting all system L transporters inhibits methionine uptake in both, cancer cells and infiltrating CD8 T cells. Nevertheless, treating tumor bearing mice with the system L transporter BCH in combination with anti-PD-L1 resulted in a synergistic effect by partly inhibiting tumor growth and increasing numbers and function of tumor-infiltrating CD8 T cells. However, further studies will have to be done to understand the underlying mechanisms for these observations.

In mammals, methionine is essential for de novo protein synthesis and required for the production of S-adenosylmethionine (SAM), a universal methyl donor required for DNA and RNA methylation and substrate for multiple histone and protein methyltransferases. Thereby, SAM links nutrient availability and cellular metabolism directly with epigenetic regulation (8, 9). Indeed, metabolomics studies performed by Bian and colleagues confirmed dramatically decreased intracellular methionine and SAM levels upon methionine starvation (3). This is in line with recent studies, which described a rapid turnover and a reduction of up to 98% of the SAM pool within hours of starving cells of methionine (8, 9). Being the methyl donor for various methyltransferases, SAM concentrations directly affect the levels of H3K79me2, an active gene mark in mammalian cells. This is mediated by DOT1L, the responsible histone methyltransferase that methylates lysine-79 of histone H3. Experiments by Bian and colleagues using genetic deletion or pharmacological inhibition of DOTL1 confirmed its crucial role in mediating the effects of methionine in CD8 T cells. Methionine starvation resulted in decreased levels of H3K79me2, which in turn, was associated with lower STAT5 expression and signaling in T cells, a pathway that is central for T cell survival and effector function. ChIP-seq experiments confirmed that the abundance of H3K79me2 in key regulatory regions of the STAT5b promoter was directly linked to methionine availability and DOT1L activity. Thus, the availability of SAM had a direct impact on T cell signalling capacity.

While Bian *et al.* unequivocally showed that diminished SAM levels led to impaired DOT1L-dependent histone methylation in CD8 T cells, it does not exclude other

epigenetic effects of methionine starvation. In line with this, a recent study described a selective loss of the histone mark H3K4me3 upon methionine starvation (8). In fact, data by Bian *et al.* (figure 2i in the article) support this finding (3); yet both studies claim effects on specific methyltransferases upon methionine restriction caused by their different affinities for SAM as a substrate. Indeed, activities of histone methyltransferases are highly sensitive to small changes in intracellular SAM concentrations, suggesting that their activity is directly regulated by SAM availability (10). In fact, DOT1L has a relatively low Km value (concentration of substrate at half maximum velocity of enzyme-mediated reaction), indicating that it only requires small amounts of SAM to become saturated suggesting that other methyltransferases with higher Km values are affected first by decreasing SAM concentrations (11). Thus, it remains unclear how substrate-regulated specificity is achieved.

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From a clinical point of view, another important question that remains to be unanswered is: should we supplement or restrict methionine in cancer patients? Bian *et al.* showed that methionine supplementation restored T cell immunity in B16F10-bearing mice and reduced tumor growth. In the same study, however, inhibition of methionine uptake with the system L transporter inhibitor BCH in combination with anti-PD-L1 therapy also reduced tumor growth. In line with a beneficial role for methionine restriction, a recent study published in *Nature* could also show slower tumor growth when mice were fed a methionine restricted diet (12). Future studies will have to address how amino acid metabolism can be manipulated to benefit cancer immunotherapies.

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Figure caption

Figure 1. Tumor cells steal methionine from CD8 T cells. Both tumor cells and T cells express the methionine transporter SLC7A5; however, with expression of the additional methionine transporter SLC43A2 tumor cells outcompete CD8 T cells for methionine uptake in the tumor microenvironment. Lower methionine concentrations in CD8 T cells lead to reduced DOTL1 activity and lower levels of dimethylated

H3K79, an active epigenetic mark. In turn, reduced H3K79 dimethylation in the *Stat5* promoter results in reduced STAT5 expression and activity.