Caloric restriction promotes the stemness and antitumor activity of T lymphocytes

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\textbf{ABSTRACT}

Recent findings have shed new light on the mechanisms through which tumor-infiltrating lymphocytes (TILs) maintain their cytotoxic potential in the context of checkpoint blockade or adoptive transfer therapies. As a consequence of the ionic unbalance occurring in the tumor microenvironment, TILs enter an adaptive caloric-restricted state, characterized by a decline in nucleocytosolic acetyl-CoA levels and induction of autophagy. These events dictate an epigenetic program that drives the acquisition of a stem-cell-like phenotype and ultimately improves antitumor function. These findings open the way to novel anticancer therapies based on the induction of autophagy by pharmacological caloric restriction mimetics.

The crosstalk between neoplastic cells and CD8\textsuperscript{+} tumor-infiltrating lymphocytes (TILs), which occurs across distinct phases of tumor progression, engenders a paradoxical condition of mutual benefit. On one hand, cancer cells are able to proliferate despite the prevalence of tumor antigen-specific TILs in the tumor bed. On the other hand, dysfunctional TILs (as generated upon persistent antigen exposure over time) can regain their oncostatic potential in the context of checkpoint blockade or adoptive transfer therapy.\textsuperscript{1,2} While this latter effect was attributed to terminally differentiated effector T cells (Teff) expressing immunosuppressive markers at their surface,\textsuperscript{3} it has recently been disclosed that a particular TIL clonotype (defined by the expression of the Tcfr transcription factor and a high capacity of self-renewal) is actually responsible for the successful outcome of checkpoint blockade and therapies based on the adoptive transfer of TILs.\textsuperscript{4,5} In this scenario, the evolutionary forces and signals that drive TILs towards the acquisition of a stem-cell-like phenotype remain largely undefined.

In their recent work, Nick Restifo’s team from the National Cancer Institute provides an integrated description of the phenotype and ultimately improves antitumor function. These findings open the way to novel anticancer therapies based on the induction of autophagy by pharmacological caloric restriction mimetics.

Importantly, Restifo’s team reports that elevated levels of [K\textsuperscript{+}]	extsubscript{e} dramatically reduced the capacity of TILs to take up nutrients from TME.\textsuperscript{6} While this effect further unbalanced the metabolic competition within TME in favor of cancer cells, it also imposed a state of adaptive caloric restriction (CR) on TILs.\textsuperscript{6} At odds with the common view that CR of T cells would be immunosuppressive,\textsuperscript{7} strategies based on reduced nutritional intake (including treatment with putative caloric restriction mimetics [CRMs] such as rapamycin and metformin) are associated with enhanced stemness of bone marrow lymphoid progenitors,\textsuperscript{8} contributing to the acquisition of a memory phenotype by favoring the glycolysis–OXPHOS switch\textsuperscript{10} and reducing immune senescence.\textsuperscript{11} Accordingly, T cells that were conditioned in vitro with [K\textsuperscript{+}]	extsubscript{e} displayed a prominent starvation-like metabolic signature, as defined by a reduction in glycolytic substrates, a drop in methionine levels, and a selective enrichment of ethanamine-derived phospholipids. Notably, similar alterations in the intracellular metabolome have previously been associated with extended organismal longevity and improved cellular fitness as they promoted autophagy.\textsuperscript{12,13} As formerly reported for CD45\textsuperscript{+} circulating leukocytes from mice or human volunteers undergoing fasting,\textsuperscript{14} elevated [K\textsuperscript{+}]	extsubscript{e} was able to stimulate autophagy and to sustain improved mitochondrial function in TILs in vitro.

Nucleocytosolic acetyl CoA (AcCoA) acts as a central metabolic inhibitor of the autophagic process.\textsuperscript{15} In nutrient-enriched settings, elevated levels of AcCoA are sensed by the acetyltransferase EP300, which mediates the inhibitory acetylation of essential autophagic machinery proteins.\textsuperscript{15} Conversely, maneuvers that reduce AcCoA biosynthesis or favor protein deacetylation (either by inhibiting protein acetyltransferases or by activating protein deacetylases) trigger cytoprotective autophagy and faithfully mimic nutrient-restricted conditions.\textsuperscript{16,17} Vodnala and colleagues report that
TILs exposed to [K+]e present a significant decrease in the nucleocytosolic (but not mitochondrial) AcCoA pool, mirrored by a reduction in protein acetylation levels. Changes in nucleocytosolic AcCoA levels directly influence cellular fate as they determine (through histone acetylation) the degree of the permissiveness of transcriptional machinery to euchromatin regions. Combined chromatin immunoprecipitation (ChIP) and deep sequencing (ChIPseq) analysis of H3K9Ac and H3K27Ac histone marks (which are associated with elevated gene transcription) revealed that [K+]e-conditioned TILs exhibit reduced H3 acetylation in several genes involved in T cell activation (such as Ifng) or exhaustion (Pdcd1, Hauvc2, Klf4), indicating that Teff functions were silenced in this setting. Bulk RNA-Seq analysis performed in similar experimental conditions found elevated mRNA levels of T cell stemness-related genes (including Bach2, Bel6, and Klf2). Of note, gene set enrichment analysis confirmed that the transcriptional signature induced by [K+]e-induced CR resembles that of the CD27+ CD62L+ Tcē7+ subset of CD8 T cells, which retains stem cell features and proficiently responds to PD1+ therapy. These results support the idea that CR (mainly acting through the AcCoA-autophagy axis) elicits epigenetic changes in T cells that are compatible with multipotency and improved memory responses. Although AcCoA depletion appears to be the major determinant of these features, it cannot be ruled out that additional metabolites contribute to shape a stem-cell-like features in CR TILs. De facto, reduction in the methyl group donor methionine (a maneuver per se able to trigger autophagy) was associated with decreased methylation of the repressive histone mark H3K27me3 in correspondence of stemness-associated loci.

To extend the relevance of these findings to preclinical settings, the authors challenged TILs isolated from patient tumor with [K+]e in vitro and found an increased proportion of CD62L+ TILs compared to their non-conditioned counterparts. In addition, [K+]e-conditioned mouse T cells specific for a melanoma antigen showed enhanced antitumor activity when adoptively transferred into B16 melanoma-bearing hosts.

In order to decipher the actual contribution of autophagy to the acquisition of a stem-cell-like phenotype, the authors demonstrated that direct administration of the AcCoA-replenishing metabolite acetate (whose metabolism is specifically altered in neoplastic conditions) 10 to [K+]e-conditioned T cells reversed the epigenetic and phenotypic changes attributed to caloric restriction and resulted in loss of multipotency features. Notably, this effect was fully recapitulated by the genetic ablation of the autophagy essential gene Atg7, confirming that autophagy determines the establishment of T cell stemness. Conversely, treatment of T cells with hydroxycitrate (HC), an inhibitor of ATP citrate lyase (ACLY) that reduces the nucleocytosolic pool of AcCoA, ignites autophagy in vitro and in vivo and enhances antitumor immunosurveillance in various types of cancers,15,20 recapitulated the epigenetic changes attributed to elevated [K+]e and restored stem-cell-like characteristics. T cells expressing a TCR specific for Hgp100 treated in vitro with HC and then adoptively transferred into mice infected with an Hgp100-expressing vaccinia virus persisted much longer than their untreated counterparts. Moreover, in vitro treatment of tumor antigen-specific T cells with HC before their adoptive transfer into tumor-bearing mice favored their capacity to reduce tumor burden and improve host survival. Based on these findings, it is tempting to speculate that the potent antitumor effect elicited by systemic administration of HC to tumor-bearing animals reflects the induction of autophagy in both neoplastic and immune cells including T lymphocytes.

Prolonged CR favors the catabolism of acetate. Accordingly, durable exposure of T cells to [K+]e resulted in the upregulation of AcCoA synthase short-chain family member 1 (Acsc1), the enzyme that converts acetate into AcCoA. Moreover, transfection–enforced overexpression of Accs1 in T cells (which leads to an increase in the mitochondrial AcCoA pool, yet does not affect the nucleocytosolic AcCoA concentration) drove the metabolic reprogramming of calorically-restricted T cells towards OXPHOS, assisted them in the acquisition of a stem-cell-like phenotype, and triggered autophagy.

In conclusion, this work sheds new light on the role of the AcCoA-autophagy axis in regulating cell fate and fitness of T lymphocytes. It will be important to determine whether other agents recently classified as CRMs (including the EP300 inhibitors spermidine and aspirin22,23) are also able to enhance the stemness and cytotoxic activity of T cells, thus improving antitumor immunity. Along similar lines, administration of the anti-aging compound ethanalamine or methionine restriction (alone or in combination with current immunotherapies) might be effective in restraining tumor progression. Beyond these speculations, the results obtained by Restifo’s team add a further level of complexity to the debate about the therapeutic roles of autophagy modulation (Figure 1). Based on the published findings, it can be predicted that systemic inhibition of autophagy must impair the acquisition of stemness required for the optimal response to immunotherapy by checkpoint blockade or adoptive transfer of tumor antigen-specific T cells. To solve this conundrum, it will be primordial to perform sophisticated high-throughput immunophenotyping experiments on TILs recovered from mice that have been exposed to pharmacological autophagy inducers and inhibitors.

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Disclosure of Potential Conflicts of Interest

GK is a co-founder of Samsara Therapeutics.
Figure 1. Non-cell autonomous effects of autophagy inducers in cancer therapy. Treatment of tumor-bearing mice with autophagy inducers (including fasting or caloric restriction mimetics [CRMs]) impinges on both malignant cells and cells from the immune system. Autophagy stimulation can be combined with distinct antineoplastic therapies (including chemotherapies inducing immunogenic cell death [ICD] chemotherapy, immune checkpoint blockade, and adoptive cell transfer [ACT]) to achieve durable anticancer immunosurveillance. DAMPs, damage-associated molecular patterns; APCs, antigen-presenting cells.

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