Domanda estratta n. 3

OGGETTO: AVVISO PUBBLICO, PER TITOLI E COLLOQUIO, PER L'ASSUNZIONE A TEMPO DET. DI N. 1 RISORA NEL PROFILO DI RICERCATORE SANITARIO – CATEGORIA DS – NELL'AMBITO DEL PROGETTO PNRR-MCNT1-2023-12377772, P.I. DR.SSA LORENZA LANDI – CUP MASTER H53C240002700

DOMANDE ARGOMENTO SPECIFICO

1. Discuti, con degli esempi, come i cambiamenti metabolici tipici delle cellule neoplastiche contribuiscono alla progressione tumorale

2. Discuti come le alterazioni del metabolismo, nel microambiente tumorale,, possono influenzare la funzionalità delle cellule immunitarie e la risposta anti-tumorale

3. Descrivi i principali modelli preclinici non animali in grado di ricapitolare le funzioni biologiche complesse tipiche del microambiente tumorale

DOMANDE INFORMATICA

1 Cos'è Excel

2 Cos'è Power Point

3 Cos'è un Database

Istituti Fisioterapici Ospitalieri - ROMA

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Article

Redirecting glucose flux during *in vitro* expansion generates epigenetically and metabolically superior T cells for cancer immunotherapy

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SUMMARY

Cellular therapies are living drugs whose efficacy depends on persistence and survival. Expansion of therapeutic T cells employs hypermetabolic culture conditions to promote T cell expansion. We show that typical *in vitro* expansion conditions generate metabolically and functionally impaired T cells more reliant on aerobic glycolysis than those expanding *in vivo*. We used dichloroacetate (DCA) to modulate glycolytic metabolism during expansion, resulting in elevated mitochondrial capacity, stemness, and improved antitumor efficacy in murine T cell receptor (TCR)-Tg and human CAR-T cells. DCA-conditioned T cells surprisingly show no elevated intratumoral effector function but rather have improved engraftment. DCA conditioning decreases reliance on glucose, promoting usage of serum-prevalent physiologic carbon sources. Further, DCA conditioning promotes metabolic flux from mitochondria to chromatin, resulting in increased histone acetylation at key longevity genes. Thus, hyperglycemic culture conditions promote expansion at the expense of metabolic flexibility and suggest pharmacologic metabolic rewiring as a beneficial strategy for improvement of cellular immunotherapies.

INTRODUCTION

Immunotherapy has dramatically altered the landscape of cancer treatment. Although immune checkpoint blockade aims to potentiate activity of endogenous tumor-reactive lymphocytes to improve tumor clearance, these therapies require a population of dormant tumor-reactive T cells capable of being re-invigorated.[1,2] [Adoptive cell therapies (ACTs), in contrast, rely on the infusion of functional tumor-specific T cells. To accomplish this, some forms of ACT (CAR-T cell therapy and T cell receptor [TCR] transgenic T cell therapy) genetically re-direct T cells from the patient's peripheral blood to recognize tumor-associated antigens. Tumor infiltrating lymphocyte (TIL) therapy, another form of ACT, directly expands lymphocytes harvested from the patient's tumor tissue. Common to all forms of ACT is *in vitro* culture, in which mitogenic stimuli and cytokines are used to generate high numbers of tumor-reactive T cells for reinfusion.] 1

[Although ACT has achieved some success in liquid tumors,[3–7] resistance is common[8,9] and solid tumors remain resistant to cellular therapies.[10,11]

Several hypotheses aim to explain why solid tumors are resistant to ACT,[9,12] but one major factor is the detrimental effect of the tumor microenvironment (TME) on T cell metabolism,[13,14] function,[15,16] and persistence.[17,18] Elevated tumor cell metabolism yields a nutrient-poor milieu,[19] which, with sustained TCR stimulation, causes T cell-intrinsic metabolic and functional aberrations, ultimately leading to reduced tumor clearance.] 2 Some recent ACT improvement strategies have focused on metabolically bolstering T cells within the TME[10,20]; however, adoptively transferred T cells also need to persist in circulation, lymphoid organs, and peripheral tissues to promote a self-renewing memory response and guard against tumor recurrence.

In addition to mitogenic stimuli and cytokines, current *in vitro* culture strategies for expanding tumor-reactive T cells rely on

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supraphysiologic levels of glucose and other metabolites—the central idea being that "more is better." We and others hypothesize these conditions do not prepare T cells for *in vivo* environments, whether in the nutrient-restricted tumor or even the periphery. Data showing considerable metabolic divergence between T cells *in vitro* and *in vivo* support this notion.[21] This led us to hypothesize that the hypermetabolic (most notably hyperglycemic) conditions in which we expand T cells for therapy may generate stresses ultimately hindering their therapeutic efficacy. The *in vitro* expansion step of ACT, often viewed as an "opportunity" for engineering, may also represent a liability.

Here, we identify glucose usage as one major divergence between T cells expanding *in vitro* versus those expanding *in vivo*. We implicate the glycolytic gatekeeper enzyme pyruvate dehydrogenase kinase 1 (PDHK1,[22] typically activated upon T cell activation[23]) as a major metabolic node driving sustained aerobic glycolysis of *in vitro*-expanded T cells. By inhibiting PDHK1 after activation but during *in vitro* expansion, the metabolic and functional capacity of ACTs can be dramatically improved.

RESULTS

In vitro-expanded T cells diverge metabolically from *in vivo*-expanded T cells

[A major barrier to adoptive T cell therapies is an inability to persist after infusion—a characteristic necessary for long-term efficacy.[24,25] *In vivo*, T cells respond to antigen and can persist an entire lifetime (and beyond[26]) as memory T cells, yet T cells activated *in vitro* only survive in culture for a few weeks[27]—suggesting a large gap between the potential of T cell longevity versus what is currently possible in culture. We thus sought to determine the metabolic features separating these two growth environments to better understand the shortcomings of *in vitro* expansion of T cells.] Ovalbumin (OVA)-specific OT-I T cells were either activated *in vitro* with cognate peptide or transferred into congenically mismatched hosts infected with vaccinia virus expressing OVA (VV-OVA) (Figure 1A). Aside from higher CD25 expression *in vitro* (likely induced by *in vitro* treatment with interleukin [IL]-2), these cells display similar levels of activation markers (Figures S1A and S1B) and expand at comparable rates (Figure S1C). However, T cells activated *in vivo* differ strikingly from their *in vitro* counterparts when assayed metabolically. By day 3 of expansion, OT-I T cells expanding *in vivo* show significantly elevated mitochondrial mass (Figure 1B), mitochondrial reactive oxygen species (indicative of mitochondrial function) (Figure 1C), and tended to have higher accumulation of neutral lipid (Figure S1D). Of note, both *in vivo*- and *in vitro*-expanded T cells shared the same capacity to take up glucose, as measured by uptake of the fluorescent glucose analogs 2-NBDG (Figure 1D) or 1-amino-Cy3-glucose (glucose-Cy3) (Figure S1E). We then analyzed previously published proteomic data comparing *in vitro* versus *in vivo* stimulation,[21] revealing several proteins involved in glycolysis (Hk1, Hk2, Pgam1, Pgk1, and Slc2a1) (Figure S1F) are expressed at lower levels during *in vivo* expansion. *In vivo*-expanded T cells also look similar in their memory formation but are more stem-like during expansion (Figures S2A–S2C). By the end of a 7-day expansion, extracellular flux analysis revealed that OT-I T cells activated *in vivo* had an elevated mitochondrial profile (Figure 1E); but, strikingly, T cells that were expanded *in vivo* were far less likely to perform aerobic glycolysis, the act of fermenting glucose into lactate under normoxic conditions (Figure 1F). A ratio of oxygen consumption rate (OCR) to glycolytic rate (extracellular acidification rate [ECAR]) further supports the reliance on oxidative metabolism rather than glycolytic metabolism of *in vivo*-expanding T cells (Figure 1G). Confocal microscopy of *in vivo*-stimulated T cells at the end of expansion shows increased mitochondrial staining (Tomm20), confirming their elevated mitochondrial metabolism (Figure 1H). Thus, once T cell activation is complete, T cells expanding *in vitro* ferment glucose into lactate far more readily than those expanding *in vivo*.

Most T cells for therapy are stimulated with some combination of TCR triggering, CD28 costimulation, and hyper-physiologic concentrations of IL-2, then expanded in media far exceeding physiologic or tumoral[28] glucose concentrations (Figure 1I). We and many others have shown that these stimuli can induce signaling pathways promoting glucose fermentation in a post-translational, translational, and transcriptionally dependent manner.[23,29,30] Indeed, activating T cells for 48 h in combinations of these stimuli revealed that each promotes aerobic glycolysis, with the combination of all three (typical stimulatory conditions) resulting in the highest level of aerobic glycolysis (Figure 1J). Thus, T cells have distinct metabolic phenotypes when activated *in vitro* versus *in vivo*, and the methods by which we propagate T cells *in vitro* promote the aerobic fermentation of glucose into lactate.

Redirecting glucose by inhibiting pyruvate dehydrogenase kinase elevates mitochondrial capacity

We have previously shown that signals originating proximally at the TCR can redirect glucose flux via phosphorylation and activation of PDHK1, which inhibits pyruvate dehydrogenase-mediated conversion of pyruvate to acetyl-coenzyme A (CoA).[23] PDHK1 activity during T cell activation thus prevents pyruvate uptake into the mitochondria, resulting in its conversion to lactic acid.[23]

PDHK1 can be inhibited using dichloroacetate (DCA),[31,32] previously used to treat lactic acidosis.[33] Although we have previously shown that *during* activation, PDHK1 activity is necessary to induce glycolysis to support effector functions,[23] we hypothesized that DCA could be repurposed *after* activation and during expansion to dampen the signals promoting excess aerobic glycolysis in culture, while preserving the cells' ability to produce glycolytic intermediates for use in cellular processes necessary for expansion (Figure 2A). In other words, by redirecting glucose into mitochondria during *in vitro* expansion, we aim to induce a more *in vivo*-like phenotype and promote mitochondrial number, health, and respiratory capacity—all features of long-lived cells.[34,35] We confirmed that DCA acutely shifts T cell glycolytic flux by assaying lactate production through ECAR (Figure S3A) or the amount of uniformly labeled ^{13}C-glucose converted to M + 3 lactate (Figure S3B) or M + 2 acetyl CoA (Figure S3C) post-acute DCA treatment. Additionally, we have previously shown that DCA accomplishes this reduction in lactate production through direct inhibition of PDHK1.[23] Thus, we aimed to condition therapeutic T cells in the presence of DCA in order to potentially generate a more "*in vivo*-activated"-like state and enhance efficacy.