Disrupting a core metabolic process in T cells may improve their therapeutic efficacy

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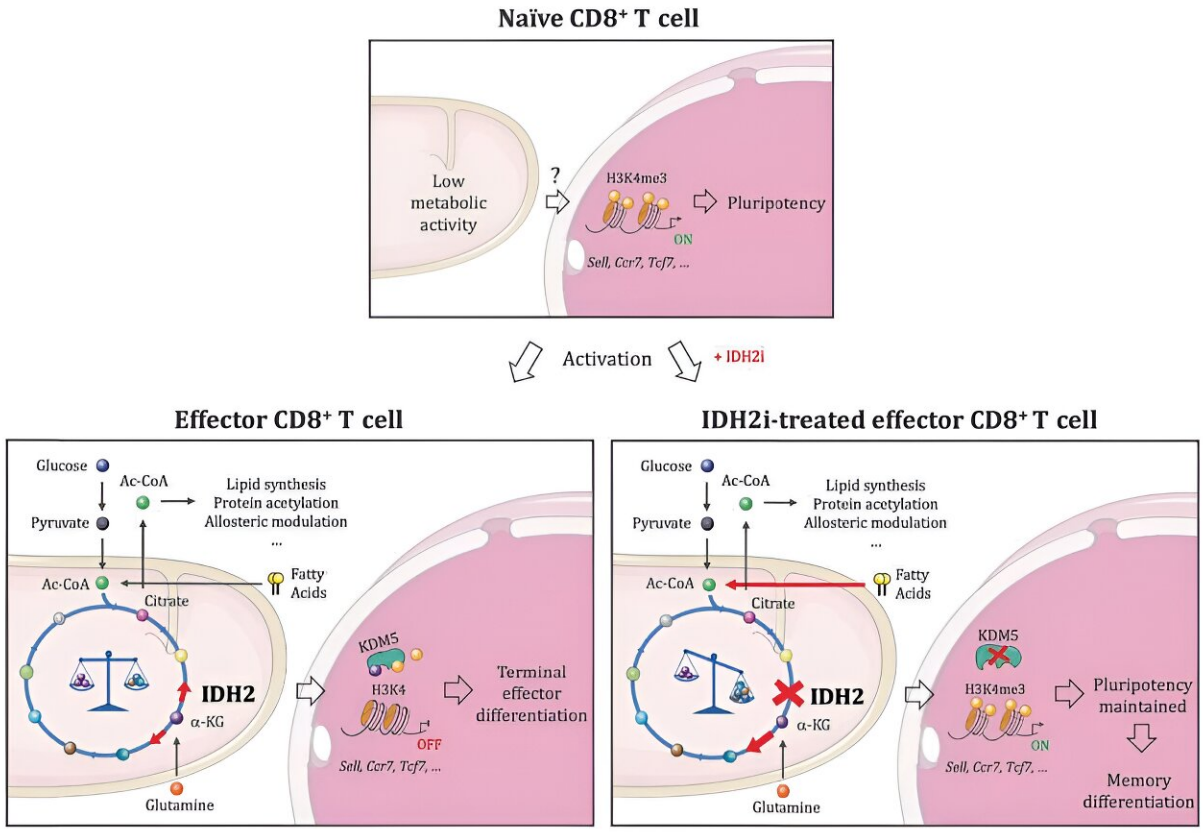

Pluripotent naive CD8$^+$ T cells are characterized by low metabolic activity and permissive H3K4me3 deposition at pro-memory genes, including *Sell*, *Ccr7* and *Tcf7*. After activation, T_E cells increase glutamine metabolism along both the oxidative and reductive pathways (represented by two red arrows on the left panel of the figure). RC generates citrate and acetyl-CoA, which is a key metabolite supporting cell growth and function. This specific T_E cell metabolism creates a unique metabolite composition (represented by balanced levels of α-

KG on one side and succinate, fumarate, malate and 2-HG on the other side) enabling the activity of the α-KG-dependent histone demethylase KDM5. KDM5-mediated demethylation of H3K4me3 in activated T cells induces a repressive chromatin state at pro-memory genes, which prevents the maintenance of pluripotency and facilitates terminal effector differentiation. Blocking RC through inhibition of IDH2 does not hinder T cell proliferation and function, as compensatory fatty acid oxidation can refuel acetyl-CoA pools, with the support of glutamine anaplerosis, which is now redirected entirely in the oxidative branch of the TCA cycle (represented by the larger red arrow on the right panel of the figure), altogether enhancing cellular OCR. This leads to a perturbation of the metabolite balance by increasing the levels of the TCA intermediates succinate, fumarate, malate and 2-HG (represented by a shift in the balance upon IDH2 inhibition). The accumulation of these inhibitory metabolites impairs KDM5 demethylase activity, and elevated H3K4me3 is likely to foster a permissive chromatin state at pro-memory genes, allowing for the maintenance of pluripotency and memory differentiation. Credit: *Nature* (2023). DOI: 10.1038/s41586-023-06546-y

In exploring an aspect of how killer T cells generate the raw materials required for their proliferation, a Ludwig Cancer Research study has uncovered an unexpected link between the immune cells' metabolism, regulation of gene expression, persistence and functional efficacy that could be exploited using existing drugs to improve cancer immunotherapy.

Researchers led by Ludwig Lausanne's Alison Jaccard and Ping-Chih Ho along with their University of Lausanne colleagues Mathias Wenes and Pedro Romero were exploring how proliferating T cells in the low-oxygen environment of tumors make citrate, a molecule essential to manufacturing membranes, which are required in large quantities to make new cells.

The question was whether the killer ($CD8^+$) T cells—which destroy sick

and infected cells—use a trick employed by multiplying cancer cells to ensure a steady supply of the critical molecule in similarly hypoxic conditions: shunting the amino acid glutamine through a series of chemical reactions, including one known as reductive carboxylation, to make citrate.

"We show in this study that $CD8^+$ T cells indeed engage this metabolic pathway, and that an extensively characterized metabolic enzyme known as isocitrate dehydrogenase plays a central role in the process," said Ho.

"But what really surprised us—and could be significant for cancer immunotherapy—is that genetically or pharmacologically disrupting the enzyme did not hamper the proliferation or function of $CD8^+$ T cells, as we expected it would. Rather, it led to the transformation of their progeny into memory T cells that are more vigorous and live far longer than their predecessors."

The researchers further report in the current issue of Nature that both mouse and human chimeric antigen receptor (CAR) T cells grown in the presence of drugs that inhibit isocitrate dehydrogenase (IDH2) take on all the key properties of memory T cells. They also demonstrate that these CAR-T cells—which are engineered to target specific markers on cancer cells—show enhanced anti-tumor activity in mouse models of melanoma, leukemia and multiple myeloma.

"When $CD8^+$ T cells are prepared for treatments involving adoptive cell transfer, they tend to be relatively exhausted, meaning they have a limited ability to proliferate and die pretty quickly after activation," said Jaccard. "This results in their inefficient engraftment and cancer recurrence. But memory T cells persist and can proliferate over and over again when activated by their targets, making them much better instruments of CAR-T and other adoptive cell therapies."

Analysis of how disrupting IDH2 activity affected the T cells revealed a link between an altered profile of metabolites in them and epigenetic regulation of their gene expression, in which the chemical tagging of DNA and its protein packaging dynamically alters chromosome structure to determine the availability of genes for reading.

"Our studies showed that when IDH2 is inhibited by drugs, the cell engages alternative metabolic pathways to compensate," said Jaccard. "That naturally alters the types and amounts of metabolites generated in the cell, and some of the metabolites affected by these changes are involved in regulating epigenetic enzymes. This is what drives the transformation of $CD8^+$ T cells into memory cells."

Specifically, IDH2 inhibition affects a core metabolic process known as the TCA cycle, forcing the T cells to activate compensatory metabolic pathways. This alters the profile of metabolites in the cells, boosting the levels of molecules that inhibit an epigenetic enzyme known as KDM5 and so changing the deposition of a key epigenetic "mark" on their chromosomes.

As a result, the chromosomes open up in a way that gives the cells' gene expression machinery access to genes that define memory T cells, triggering their transformation. In the absence of IDH2 inhibition, those genes are kept under wraps, bolstering the terminally exhausted $CD8^+$ T cell identity.

Aside from their practical applications, these findings indicate that reductive carboxylation is not required for the proliferation of $CD8^+$ T cells, as it appears to be for rapidly growing cancer cells. Rather, the metabolic process switches on gene expression programs that lock the immune cells into a terminal effector state—one in which they retain functional capability but their lifespans and proliferative capacity are curtailed.

Ho says his team now plans to examine how best to sabotage reductive carboxylation to prepare better cells for CAR-T and other adoptive T cell therapies, comparing pharmacologic inhibition with gene editing strategies to that end. He and his colleagues will also explore whether cancer cells adapt to hijack the metabolism and epigenetic regulation of T cells to push them into a less persistent, terminal effector state.

More information: Alison Jaccard et al, Reductive carboxylation epigenetically instructs T cell differentiation, *Nature* (2023). DOI: 10.1038/s41586-023-06546-y

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