CD8⁺ TILs enhance PPAR α signalling and FA catabolism to partially preserve effector functions

IMMUNOTHERAPY

CD8⁺ T cells — burn fat, get fit

How tumour-infiltrating T lymphocytes (TILs) adapt to metabolic challenges in the tumour micro-environment (TME) that might lead to their exhaustion — such as hypoglycaemia and hypoxia — to preserve their antitumour functions is not well understood. Now, a study published in *Cancer Cell* reports that CD8⁺ TILs facing these dual metabolic constraints enhance peroxisome proliferator-activated receptor α (PPAR α) signalling and switch to fatty acid (FA) catabolism to preserve effector functions and, demonstrates that a PPAR α agonist improves tumour growth control and synergizes with programmed death protein 1 (PD1) blockade in melanoma mouse models.

Normal and B16-*Braf*^{V600E} tumour-bearing mice were given a mixture of two adenovirus-based vaccines to stimulate both melanoma-associated antigen (MAA)-specific and human papilloma virus E7 (E7)-specific CD8⁺

T cell responses. Although vaccination delayed tumour progression, at day 30, MAA- and E7-specific CD8⁺ TIL numbers declined by ~90% and ~80%, respectively, and displayed increased expression of co-inhibitors, including PD1 and lymphocyte activation gene 3 protein (LAG3), and decreased production

of effector molecules. As CD8⁺ TILs became functionally exhausted regardless of their antigen specificity, the authors speculated that additional factors beyond continued antigen stimulation drive exhaustion. Accordingly, upregulation of hypoxia-inducible factor 1 α (HIF1 α) was reported in MAA- and E7-specific CD8⁺ TILs, which suggests their exposure to hypoxia. Indeed, HIF1 α knockdown in CD8⁺ T cells both *in vitro* and *in vivo* mechanistically demonstrated that HIF1 α increases LAG3 expression, but not PD1, and impairs T cell effector function. Interestingly, CD8⁺ TILs with HIF1 α knockdown increased and decreased expression of glycolysis-related and tricarboxylic acid (TCA) cycle-related genes, respectively, and increased expression of PPAR α , a key transcription factor involved in FA catabolism.

Next, CD8⁺ T cell metabolism was characterized *in vivo* by analysing the intensity of isotope-labelled metabolites using liquid chromatography-mass spectroscopy. In vaccinated tumour-bearing mice given [¹³C] glucose or [¹³C] palmitate, the intensity of glycolysis metabolites declined at day 30, whereas FA catabolism metabolites increased. Levels of free FAs were consistently elevated during tumour progression in the interstitial fluid of B16-*Braf*^{V600E} tumours, melanoma patient-derived xenograft (PDX) tumours and, importantly, in human melanoma metastases compared with human sera. Strikingly, CD8⁺ TILs isolated from resected melanoma metastases showed enhanced FA uptake and higher PPAR α expression *in vitro* compared with circulating T cells.

As PD1 expression increased over time in CD8⁺ TILs, Zhang *et al.* then investigated whether treatment with an anti-PD1 monoclonal antibody (α PD1) altered their metabolism or function. Compared with an isotype control, α PD1 treatment delayed tumour progression in vaccinated and unvaccinated mouse models and did not affect CD8⁺ TIL differentiation, metabolism or effector function. In addition, CD8⁺ T cells derived from mice treated with fenofibrate — a PPAR α agonist that increases FA catabolism — had enhanced antitumour effects compared with controls upon transfer to vaccinated, tumour-bearing mice. Indeed, analysis of fenofibrate-treated CD8⁺ TILs isolated 3 weeks after transfer revealed increased expression of FA catabolism genes, increased PD1 expression and greater effector functions. Crucially, treatment of recipient mice with α PD1 during transfer of fenofibrate-treated CD8⁺ T cells synergistically delayed tumour progression and completely prevented tumour growth in ~50% of mice.

Collectively, the findings demonstrate that CD8⁺ TILs enhance PPAR α signalling and FA catabolism to partially preserve effector functions in a hypoglycaemic and hypoxic TME, and that metabolic reprogramming of these cells using a PPAR α agonist improves their antitumour functionality and increases efficacy of PD1-targeted immunotherapy in melanoma. The study also warrants clinical investigations. “We would like to test if preconditioning CD8⁺ T cells towards FA catabolism prior to their transfer into patients extends T cell survival, improves their functions and, in the end, benefits patients,” concludes lead investigator Hildegund Ertl.

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ORIGINAL ARTICLE Zhang, Y. *et al.* Enhancing CD8⁺ T cell fatty acid catabolism within a metabolically challenging tumor micro-environment increases the efficacy of melanoma immunotherapy. *Cancer Cell*. **32**, 377–391 (2017)

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