IMMUNOTHERAPY

Leptin boosts T cell function in tumours

Even though the therapeutic use of oncolytic viruses has reached the clinical setting, durable remission rates in patients with melanoma are limited and the mechanisms of action remain poorly understood. Rivadeneira et al. now show that tumour responses to the oncolytic *Vaccinia* virus involve the recruitment of new but metabolically dysfunctional CD8$^+$ T cells and can be improved by engineered expression of leptin, acting as a metabolic reprogrammer in T cells.

The authors explored the ability of the oncolytic *Vaccinia* virus to modulate the tumour immune infiltrate and trigger an effective anti-tumour immune response. The virus was injected into established CL24 mouse melanoma cell line — derived tumours in mice (CL24 cells carry driver mutations that are common in human melanoma, and these tumours are insensitive to anti-PD1 monotherapy). Compared with PBS-injected tumours, the treated tumours showed regression but no complete responses. Single-cell RNA sequencing (sc-RNA-seq) of tumour immune infiltrates taken prior to regression from *Vaccinia*-treated mice and PBS-treated mice for comparison and subsequent flow cytometric analysis showed that *Vaccinia* infection increased infiltration of new effector-like, non-exhausted CD8$^+$ T cells, natural killer cells and monocytes. However, CD8$^+$ T cells in *Vaccinia*-treated tumours were still metabolically dysfunctional as indicated by the lack of difference in mitochondrial content and glucose uptake compared with CD8$^+$ T cells from PBS-treated tumours, likely accounting for the lack of complete responses.

As a potential option to reverse metabolic dysfunction of CD8$^+$ T cells in *Vaccinia*-treated tumours, the researchers looked into the adipokine leptin, which is known to promote an inflammatory response through metabolic reprogramming. Indeed, in vitro, leptin increased oxygen consumption and mitochondrial mass of CD8$^+$ T cells isolated from peripheral lymph nodes (LNs), and induced signalling pathways to promote mitochondrial biogenesis. These changes could be beneficial in tumour-infiltrating CD8$^+$ T cells, which express the leptin receptor at even higher levels than LN-derived CD8$^+$ T cells. Importantly, leptin receptor expression in CD8$^+$ T cells correlated with PD1 expression in tumours: it was higher in PD1-positive tumour infiltrating CD8$^+$ T cells than PD1-negative tumour infiltrating CD8$^+$ T cells and LN-derived PD1-negative CD8$^+$ T cells. Taking this to the in vivo setting, the researchers engineered CL24 cells to overexpress leptin (CL24^{leptin}). While their growth rate was unchanged in vitro compared with control cells, tumours derived from CL24^{leptin} cells grew slower than control tumours in mice, but only if CD8$^+$ T cells were present and not experimentally depleted. CL24^{leptin} tumours in mice that had a T cell-restricted heterozygous deletion of the leptin receptor (which is haploinsufficient) grew at a similar rate to CL24 control tumours or CL24^{leptin} tumours in CD8$^+$ T cell-depleted mice — pointing to leptin as a mediator of this effect.

> “tumours derived from CL24^{leptin} cells grew slower than control tumours in mice”

The immune infiltrate of CL24^{leptin} tumours contained a higher percentage of CD8$^+$ T cells than control tumours. Even though the expression levels of PD1 and TIM3 indicated their phenotypical exhaustion, these T cells produced higher levels of interferon-γ and tumour necrosis factor as shown in flow cytometric analysis, and were more proliferative in situ than CD8$^+$ T cells in control tumours. Also, these T cells had a metabolic profile similar to that of leptin-stimulated T cells from previous analyses, underlining the metabolic reprogramming and T cell activating effects of leptin.

In view of therapeutic applications, the authors modulated the oncolytic *Vaccinia* virus to express leptin (VV^{leptin}), which could be used to induce leptin expression in established CL24 tumours in mice. Here, a single VV^{leptin} intratumoural injection led to larger regressions than control *Vaccinia*-treated tumours, and increased the proportion of complete responses. VV^{leptin} injection increased CD8$^+$ T cell activation, as well as mitochondrial capacity in these T cells, but did not affect the numbers of infiltrating T cells compared with *Vaccinia*-treated tumours, showcasing the phenotypic change induced by leptin. Furthermore, sc-RNA-seq analysis of the immune infiltrate showed that VV^{leptin} treatment increased effector memory and memory signatures in tumour infiltrating T cells — this memory response was reflected in tumour rejection when VV^{leptin}-treated tumour-bearing survivors were rechallenged.

Of note, injections of control *Vaccinia* or VV^{leptin} into CL24- tumours in obese mice, which have chronically increased levels of leptin, did not show leptin-mediated benefits in controlling tumour growth, likely owing to leptin resistance in obesity.

These results also highlight the dual application of oncolytic viruses as an immune-modulating therapy and drug delivery system.

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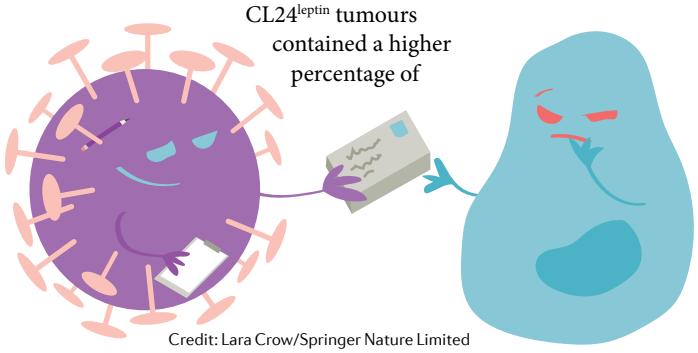

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ORIGINAL ARTICLE Rivadeneira, D. B. et al. Oncolytic viruses engineered to enforce leptin expression reprogram tumor-infiltrating t cell metabolism and promote tumor clearance. *Immunity* https://doi.org/10.1016/j.immuni.2019.07.003 (2019)

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