IMMUNOLOGY

Tumour-reactive T cells work remotely using IFNγ

Following antigen encounter, tumour-reactive CD8$^+$ T cells release cytokines, including interferon-γ (IFNγ). IFNγ signalling can increase programmed cell death ligand 1 (PDL1) as well as major histocompatibility complex class I (MHC-I) expression in cancer cells, but IFNγ also modifies stromal cells in the tumour microenvironment (TME). Although it is well established that IFNγ is secreted by T cells in a directional manner towards the immunologic synapse, in vitro data suggest that IFNγ activity may not be restricted to that. So far, the extent of its spatiotemporal activity in vivo remains unclear. Now, two groups report in *Nature Cancer* that IFNγ secreted by tumour-reactive T cells diffuses into the TME and acts on remote tumour cells to modify tumour behaviour.

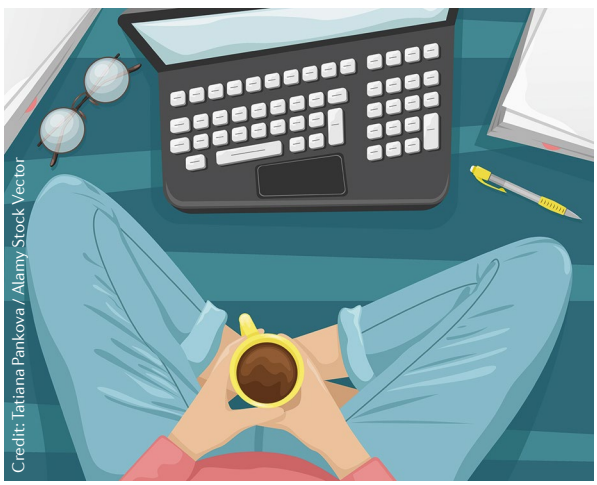
Hoekstra et al. used the IFNγ-sensing reporter (IGS), an IFNγ-signalling responsive promoter that drives expression of the Katushka fluorescent protein, to monitor IFNγ signalling in vivo and specifically the IFNγ responsiveness of those cancer cell populations that were not directly recognized by tumour-reactive CD8$^+$ T cells (bystander cells). Mosaic tumours, composed of OVCAR5 ovarian cancer cells expressing the patient-derived mutant cyclin-dependent kinase 4 (CDK4R>L) neoantigen (Ag), plus Ag$^-$OVCAR5 cells that expressed the IGS reporter, were grown in immunodeficient mice. After treatment with Ag-specific CD8$^+$ T cells, ~64% of bystander cells activated IFNγ signalling, whereas only ~3% of Ag$^-$ cells in control mice showed active IFNγ signalling. When tumours were composed of relatively fewer Ag$^+$ cells (10%) compared with Ag$^-$ cells (90%), ~30% of Ag$^-$ cells activated Katushka following Ag-specific T cell transfer, whereas the signal was lost in mixed tumours containing Ag$^-$ cells lacking the IFNγ receptor gene (*IFNGR*).

> IFNγ diffused across the tumour instead of acting on a limited number of tumour cells

Intravital imaging of mosaic tumour areas showed that the response of bystander cells peaked at 120 h after T cell transfer. Analysis of tumours with large and strictly defined islands of Ag$^+$ or Ag$^-$ cells showed that Ag-specific T cells located preferentially to Ag$^+$ areas, and that bystander cells more than 800 µm away from the site of Ag–T cell interaction still responded to IFNγ.

Phenotypically, bystander cells increased PDL1 expression, and underwent cell death in vitro, which was dependent on *IFNGR* expression. In tumours composed of *IFNGR*$^+$ or *IFNGR*$^-$ bystander cells and Ag$^+$ cancer cells, Ag-specific T cell transfer led to *IFNGR*$^+$ but not *IFNGR*$^-$ bystander cell depletion. In addition, tumours containing *IFNGR*$^-$ bystander cells grew more slowly after T cell transfer compared with tumours containing *IFNGR*$^+$ bystander cells.

Thibaut et al. analysed mosaic tumours of *Myc*-driven B cell lymphoma cells, in the bone marrow of *Rag2*$^{-/-}$ mice (which cannot generate mature T and B cells), using ovalbumin (Ova) as Ag expressed in a subpopulation of lymphoma cells coupled with fluorescent protein expression. Ova-specific (OT-I) CD8$^+$ T cells, which these mice were treated with, accumulated in Ova$^+$ tumour areas, whereas Ova$^-$ areas did not have prolonged T cell–tumour interactions. However, MHC-I and PDL1 expression, used as a proxy for IFNγ signalling activation, increased to a similar extent in both Ova$^+$ and Ova$^-$ regions. When the number of transferred OT-I T cells was reduced tenfold, MHC-I upregulation was reduced homogeneously, suggesting that IFNγ diffused across the tumour instead of acting on a limited number of tumour cells, in line with the observations by Hoekstra et al.


Credit: Tatiana Pankova / Alamy Stock Vector

To analyse the dynamics of the response to IFNγ, Thibaut et al. generated a construct of a STAT1–GFP fusion protein combined with a nuclear mCherry protein (cnS1) and expressed it in H-Y$^+$ lymphoma cells, to observe STAT1 nuclear translocation as a reporter of active cytokine signalling. In cnS1$^+$H-Y$^+$ lymphoma-bearing *Rag2*$^{-/-}$ mice, two-photon imaging showed that transfer of H-Y-specific CD8$^+$ T cells led to nuclear STAT1 in lymphoma cells, irrespective of T cell encounter. Similarly, mosaic tumours containing a mixture of Ova$^+$and Ova$^-$cnS1$^+$lymphoma cells and treated with OT-I CD8$^+$ T cells showed nuclear STAT1 in bystander cells, overall indicating that cytokine signalling activation can occur without T cell interaction.

Single cell-RNA sequencing data from immune infiltrate of eight untreated patients with melanoma showed that CD8$^+$ T cells were the main producers of IFNγ. Even though tumour cells could not be analysed due to low sample size, IFNγ-responsive gene expression in monocytes and intratumoural neutrophils was significantly correlated with the frequency of IFNγ-producing CD8$^+$ T cells, confirming IFNγ signalling in multiple cell types in the TME.

These studies demonstrate the ability of tumour-reactive CD8$^+$ T cells to modulate growth of bystander cells via IFNγ, opening questions about the cumulative cytokine-mediated remote activity of T cells in the TME.

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ORIGINAL ARTICLE Hoekstra, M. E. et al. Long-distance modulation of bystander tumor cells by CD8$^+$ T-cell-secreted IFNγ. *Nat. Can.* **1**, 291–301 (2020) | Thibaut, R. et al. Bystander IFN-γ activity promotes widespread and sustained cytokine signaling altering the tumor microenvironment. *Nat. Can.* **1**, 302–314 (2020)