What's driving T cell dysfunction?

T cells exposed to continuous stimulation, such as in the cancer microenvironment or during chronic infection, become dysfunctional ('exhausted'). Although the co-inhibitory receptors that downregulate T cell activity are fairly well characterized (and hotly pursued as therapeutic targets), much less is known about their regulation and the molecular pathways underlying T cell exhaustion. Now, two reports in *Nature* identify NR4A transcription factors as key mediators of T cell exhaustion.

Liu et al. carried out a genome-wide transcriptomic and epigenetic screen of in vitro-generated tolerant CD4⁺ T cells. Unsurprisingly, they found anergy-related genes and transcriptional repressors to be upregulated, whereas effector genes and translational components were downregulated. Pathway analysis revealed a close association with NR4A1-regulated transcriptional changes, and NR4A1 itself was found to be specifically upregulated in tolerant T cells.

Further in vitro experiments showed that overexpression of NR4A1 in CD4⁺ T cells suppresses the expression of effector cytokines such as interleukin-2 (IL-2) and interferon- γ (IFN γ) and upregulates anergy-related genes. Conversely, ablation of NR4A1 in both CD4⁺ and CD8⁺ T cells considerably enhanced their capacity to produce effector cytokines and increased their proliferative capacity.

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In vivo models of oral or peptide-induced tolerance in wild-type (WT) or *Nr4a1*^{-/-} mice showed that NR4A1 is essential for the downregulation of T cell responses. Next, the authors investigated whether NR4A1 is also involved in T cell exhaustion in anticancer responses. Using a mouse model of lymphoma, they found that adoptively transferred cancer cell-specific *Nr4a1*^{-/-} CD8⁺ T cells were significantly more potent at eliminating tumours than their WT counterparts. They also expressed lower levels of inhibitory receptors such as PD1 and TIM3 and showed better tumour infiltration. Similarly, ablation of *Nr4a1* in virus-specific CD8⁺ T cells enhanced their activity, indicating that NR4A1 deficiency prevents CD8⁺ T cell dysfunction.

Next, the authors investigated the binding sites of NR4A1 in the genome. Unexpectedly, they found that a substantial number of binding sites overlapped with those for JUN, a subunit of the transcription factor AP-1. Insufficient binding of AP-1 is known to lead to T cell dysfunction, and further analyses indicated that NR4A1 mediates T cell exhaustion by antagonizing the AP-1-mediated transcriptional programme.

Meanwhile, Chen and colleagues investigated the role of NR4A transcription factors in chimeric antigen receptor (CAR) T cells. These cells have shown remarkable results for the treatment of B cell malignancies, but their development for solid tumours is more challenging, partly because they are prone to exhaustion.

Using cancer cell-specific CAR T cells in two mouse models of solid tumours, the authors observed that exhausted CAR T cells express higher

levels of the NR4A family members NR4A1, NR4A2 and NR4A3 compared with their less-exhausted counterparts. Moreover, regions of open chromatin in exhausted T cells were selectively enriched for NR4A-binding motifs. On the basis of this observation, and the finding that the expression of NR4A family members in human tumour-infiltrating CD8⁺ T cells positively correlates with PD1 and TIM3 expression, they generated NR4A triple knockout (*Nr4a* TKO) CAR T cells. These cells were much more effective at inducing tumour regression and enhancing survival compared with WT or CAR T cells with single knockouts of NR4A family members, indicating that these have partially redundant roles. The *Nr4a* TKO cells produced higher levels of effector cytokines, expressed lower levels of inhibitory receptors and had a strong enrichment in open chromatin regions with binding motifs for transcription factors involved in T cell effector functions, such as nuclear factor- κ B (NF- κ B) and AP-1.

The authors had previously shown that the transcription factor NFAT, in the absence of its activation-associated partner AP-1, can induce T cell dysfunction and propose that an NFAT–NR4A axis controls the T cell exhaustion programme. They also point out that NR4A deficiency, leading to the downregulation of PD1, is functionally similar to PD1 blockade. However, it affects a much wider range of regulatory elements, indicating that targeting NR4A family members may be a powerful anticancer approach.

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ORIGINAL ARTICLES Chen, J. et al. NR4A transcription factors limit CAR T cell function in solid tumours. *Nature* <https://doi.org/10.1038/s41586-019-0985-x> (2019) | Liu, X. et al. Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction. *Nature* <https://doi.org/10.1038/s41586-019-0979-8> (2019)

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