What's driving T cell dysfunction?

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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 IL-2 and 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.

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 co-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 than 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 than 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 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 they 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 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) | 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)

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