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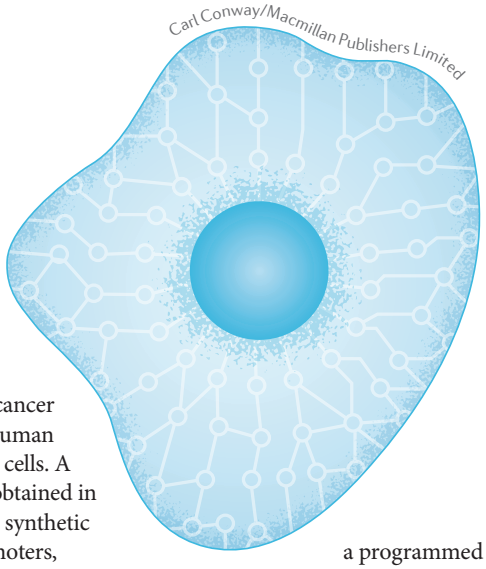
CANCER IMMUNOTHERAPY

Rewiring cancer cells



A number of factors ranging from the scarcity of tumour-specific neoantigens, tumour immunosuppression and toxicity caused by systemic delivery of immunomodulators restrict the efficiency of cancer immunotherapies. To overcome these obstacles, Nissim, Wu *et al.* have engineered a synthetic gene circuit enabling the production of combinatorial immunomodulators specifically from within cancer cells to trigger an antitumour T cell response.

Synthetic biology uses principles similar to those of electronics; gene circuits can be built based upon Boolean AND gates where an output is only generated when two inputs are simultaneously active. Using this strategy, the authors designed an RNA-based circuit consisting of two modules, each regulated by a separate synthetic cancer-specific transcription factor promoter (S(TF)p). The promoter of module 1 regulates the expression of an RNA that encodes an output protein, designed with an auto-inhibitory loop to repress its own expression. The output protein is only expressed when the promoter of module 2 is active, as it regulates a microRNA sponge that can relieve the auto-inhibition of module 1.

Testing the increased selectivity of the gene circuit to distinguish cancer cells from normal cells, they showed that the ovarian cancer-specific promoters, S(MYC)p and S(E2F1)p, exhibited 36-fold and 570-fold activation, respectively, in human OVCAR8 ovarian cancer cells over that of primary human ovarian epithelium (HOV) cells. A similarly high output was obtained in breast cancer cells by using synthetic breast cancer-specific promoters, demonstrating the adaptability of the circuit to target multiple cancer types.

> “the synthetic gene circuit could direct the immune system to kill tumour cells”

To determine whether the synthetic gene circuit could direct the immune system to kill tumour cells, immunomodulators were encoded as the output proteins. One such output was a surface T cell engager (STE) composed of a T cell co-receptor CD3 single-chain variable fragment fused to a membrane anchor. Lentiviral transduction of this circuit into cells led to robust specific T cell-mediated killing of OVCAR8 cells compared with HOV cells *in vitro*. Moreover, intraperitoneal tumour burden decreased sixfold in immunodeficient mice injected with circuit-transduced OVCAR8 cells and human T cells compared with mice injected with OVCAR8 cells in which the circuit was not induced.

Developing the circuit further to express multiple immunomodulators, the authors integrated the STE, along with CC-chemokine ligand 21 (CCL21), interleukin-12 (IL-12) and a programmed cell death protein 1 (PD1) antibody as outputs. In the same ovarian cancer model, this combination circuit (termed SCIP) reduced tumour burden to a much greater extent than the STE output alone and prolonged survival of the mice. The robust antitumour effect was also observed when only 15% of the total injected OVCAR8 cells were expressing the SCIP circuit or if the circuit was delivered directly by injection as a lentiviral mix.

As this synthetic gene circuit platform can be easily tailored, there is great promise that it could be harnessed to drive other immunomodulators and target many different cancers.

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