Biomedicine

Synthetic circuits suited for the clinic

Mohamad Hamieh & Maria Themeli

Synthetic receptor proteins can enable customized and flexible control of immune cells called T lymphocytes. A defined framework for the proteins' design now improves their potential for use in cancer immunotherapy.

A promising tool in the fight against cancer is CAR T therapy, in which immune cells called T cells are engineered to express a synthetic receptor protein, the chimeric antigen receptor (CAR), on their surface. CAR proteins recognize an extracellular target — an antigen molecule from a nearby tumour cell — and stimulate the T cell to trigger an immune cascade in response. However, the widespread application of this emerging therapy in tumours is limited by the lack of truly tumour-specific antigens, which often leads to unwanted side effects in healthy tissues. The design of synthetic-protein circuits that prevent CAR T cells from being activated outside tumours could reconcile potency with safety. Writing in *Cell*, Zhu *et al.*[1] describe one such system of proteins.

The paucity of tumour-specific antigens has prompted the development of synthetic circuits based on AND logic gates, which require input from two antigens that are tumour-specific only in combination[2]. In 2015, the group that performed the current study exploited this approach to design a circuit[3] involving a synthetic Notch (synNotch) receptor protein, and used the circuit to spatially control CAR expression and function.

SynNotch contains an extracellular antigen-binding domain and an intracellular synthetic transcription factor derived from yeast and viral proteins. The two are linked by a transmembrane domain from the mouse Notch-1 protein. Antigen engagement with the extracellular domain leads to cleavage of the transmembrane domain by a γ-secretase enzyme[3,4], releasing the transcription factor, which moves to the nucleus, binds to DNA and promotes expression of the *CAR* gene. The CAR then mediates T-cell activation after binding to a second antigen.

Activation of the synNotch circuit successfully controlled CAR-T-cell function *in vitro* and in animal models[3,5]. However, the system had drawbacks that prohibited its translation to clinical practice. For example, the receptor was highly likely to elicit an unwanted immune response (known as immunogenicity) in hosts, because of its non-human elements[3,5]. Zhu *et al.* found that a synNotch equivalent built using human Notch-1 parts had suboptimal functionality.

To address the system's limitations, they therefore revisited the original synNotch. They systematically examined sets of human-derived domains: 8 extracellular domains; 88 transmembrane domains; and 76 juxta-membrane domains (which lie between the transmembrane and transcription-factor domains, and can have a role in receptor trafficking and function). They used combinations of components to build new synthetic receptors (Fig. 1), which they dubbed synthetic intramembrane proteolysis receptors (SNIPRs), and tested the effect of each component on antigen-dependent induction of CAR expression in T cells.

The extracellular domain of synNotch contains a hinge region that connects the antigen-binding domain to the transmembrane core. The authors found that a range of hinges were compatible with SNIPR functionality, including simple repeats of the amino-acid sequence glycine–glycine–serine, as well as hinges already in use in CAR therapies, such as a truncated variant of the immune protein CD8α. For the transmembrane domain, the top performers were proteins from the Notch or calsyntenin families, which all have a carboxy-terminal glycine–valine amino-acid sequence that is needed for cleavage by γ-secretase.

Zhu and colleagues found that juxta-membrane domains that had basic amino-acid residues adjacent to the membrane conferred optimal SNIPR function, whereas acidic residues rendered the SNIPR inactive. Interestingly, they found that rational selection of different juxta-membrane domains could be used to induce varying levels of expression of a gene that encodes a signalling molecule called a cytokine. This provides proof of principle that the SNIPR system could be used for titratable local delivery of a therapeutic payload — for example, a toxin or molecule that modifies the micro-environment of the tumour.

Next, Zhu *et al.* designed humanized synthetic transcription factors to minimize immunogenicity and prevent off-target gene transcription. In each of these, the DNA-binding domain comprised either a combination of human DNA-binding domains that are absent in T cells, or synthetic 'zinc finger' domains. This DNA-binding domain was linked to a transactivation domain (a region to which other

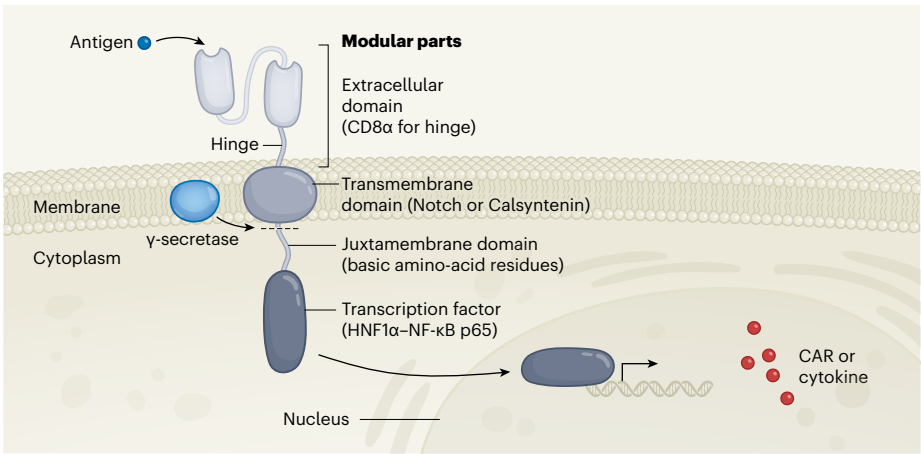

Figure 1 | A modular synthetic receptor to detect tumour antigen molecules. Zhu *et al.*[1] have built synthetic receptor proteins called synthetic intramembrane proteolysis receptors (SNIPRs). These proteins are activated by antigens on a tumour cell's surface that bind to the SNIPR extracellular domain and trigger cleavage (broken line) of the transmembrane domain by the enzyme γ-secretase. The transcription factor domain then moves to the nucleus to promote expression of a chimeric antigen receptor (CAR) protein. In turn, CAR binding by a different tumour antigen triggers a selective immune response against the tumour cells (not shown). The SNIPR can also induce expression of other molecules, such as signalling molecules called cytokines. The authors developed sets of human-derived components for every part of the protein labelled, meaning that SNIPRS can be built in a modular, customizable manner to maximize performance in a given setting. (Names in brackets indicate the proteins that produced optimal performance in the authors' analysis.)

proteins bind to help activate transcription) from a human protein called NF-κB p65.

The authors found that, of all their synthetic transcription factors, a DNA-binding domain from a liver-specific human protein called hepatocyte nuclear factor 1-alpha (HNF1α) activated gene transcription most potently in response to antigen binding. They therefore used this transcription factor as part of a fully human SNIPR for further assessment. The group demonstrated that the humanized SNIPR-to-CAR circuit has clinical potential in several mouse-tumour models – in each case, it enabled specific eradication of cells expressing both antigens required for circuit activation, and spared cells harbouring only one.

Zhu *et al.* have provided a comprehensive framework for the modular assembly of humanized SNIPRs that have the potential for clinical application in CAR-T-cell therapy. The concept could theoretically be extended to a broad range of cell types and diseases. However, the current study focused only on T cells, so the universality of the authors' design principles remains to be determined.

Notably, in certain designs, SNIPR-circuit activity was enhanced by activation of the T-cell receptor (TCR) – a surface protein responsible for T cells' normal role of triggering immune responses to foreign antigens. This property could be beneficial when the desirable output is the expression of a CAR, but could be unwanted when the local, titrated delivery of a potentially toxic therapeutic agent is necessary. In the latter case, the authors suggest that cells could be further edited to eliminate TCR expression.

Furthermore, there are still some concerns about the kinetics of CAR induction and decay – kinetics that define a CAR's ability to selectively target a tumour while protecting normal tissues. After T cells are injected systemically, they circulate in the body and localize in the tumour; induction of CAR expression needs to be fast enough to engage the tumour when T cells reach it, but the protein needs to decay before T cells begin to circulate again to other sites. The best-performing humanized SNIPR candidate induced CAR expression at levels similar to those achieved by the conventional synNotch, but significantly more slowly (over 72 hours, compared with 24 hours). As such, the SNIPR-carrying cells had lower and slower tumour-cell-killing capacity than do conventional CAR T cells.

Promisingly, no CAR expression was detected *in vivo* in tumours lacking SNIPR target antigens. However, Zhu *et al.* did not provide detailed evaluation of CAR decay data for SNIPRs compared with synNotch. In the synNotch system, decay of CAR expression was slow enough that the engineered cells activated immune responses against non-tumour cells that expressed the CAR target antigen if the non-tumour cells were in close proximity to the tumour[6]. Thus, careful antigen pairing is still required during SNIPR design, and the 'safe distance' that would protect healthy tissues from toxicity remains to be determined.

Overall, Zhu and colleagues' work demonstrates the tremendous potential of synthetic biology to control the behaviour of therapeutic cells. The SNIPR circuit has many improved features compared with its precursor. The use of building blocks of human origin reduced the protein's immunogenicity to levels comparable with a CAR currently in clinical use. This reduces the risk that the protein will be rejected by the immune system – although further assays are required to confirm this. Moreover, the humanized SNIPR's compact size compared with first-generation synNotch, its efficiency in low copy numbers, its high sensitivity to low levels of antigen and its potential for activation by a range of ligands further support its clinical potential. Together, these benefits mean that Zhu and colleagues' toolkit should help to expedite the clinical implementation of this type of synthetic circuit in cancer immunotherapy.

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Chemical biology

Synthesis provides insight into a traditional medicine

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A compound made by plants used in traditional medicine has been prepared by chemical synthesis, providing enough for biological testing. The unexpected finding that it acts at opioid receptors raises prospects for drug discovery. **See p.917**

The bark of *Galbulimima* plants is used in the traditional medicine and ritual practices of people in the Papua New Guinea region as a painkiller, fever remedy and hallucinogen[1]. The bark, sometimes together with leaves of the *Homalomena* plant, is consumed to induce visions and a dream-like state that lasts for about one hour, followed by a sense of calmness, euphoria and then drowsiness (see go.nature.com/3feq5fu). On page 917, Woo and Shenvi[2] describe a remarkably innovative and scalable approach for the preparation of GB18 – an alkaloid compound found in *Galbulimima* bark. By gaining access to this complex molecule through chemical synthesis, the authors demonstrate its previously unknown ability to act at opioid receptors. GB18 might therefore serve as a platform for the development of medicines that target these receptors.

Human medicine relies on the continual discovery and development of new types of biologically active organic molecule. The connectivity of the atoms and the corresponding 3D structure of such molecules determine pharmacological parameters such as potency, activity and selectivity for a biological target. Even small changes in the structure, such as the replacement of a single atom in a molecule, can lead to profound differences in biological activity and efficacy[3].

The field of drug discovery broadly encompasses the process of identifying and optimizing the structure of an organic molecule for use as a medicine. Synthetic organic chemistry is the technology that enables the construction of well-defined and structurally complex molecules for biological testing. Molecules isolated from living organisms – broadly known as natural products – have historically been a potent source of inspiration for organic chemists working in drug development. Between 1981 and 2019, 32% of approved small-molecule drugs had structures that were based on those found in bioactive natural products[4]. However, the typically low natural abundance of these compounds in the organisms from which they derive has necessitated the development of practical chemical syntheses to obtain sufficient quantities of complex natural products