SYNTHETIC BIOLOGY

Customizing cell-cell communication

Synthetic Notch receptors with chimeric extra- and intracellular domains can be tailored to tune cell-cell communication.

Cells communicate with each other through a variety of different signaling pathways. By tinkering with receptors at the cell surface and by modulating downstream signaling pathways, it may be possible to change a cell's behavior in response to its neighbors, which could be useful both for basic research and for translational applications.

Wendell Lim from the University of California, San Francisco, is interested in these types of manipulations. "We were looking for what kinds of systems would allow us to expand on the channels that we had for modulating and engineering new modes of cell-cell communication," he says. He and his team focused on the Notch receptor, which is used for many purposes in different tissues and which has a very direct, one-step mechanism of signal transduction to the cell nucleus. Upon activation, Notch is cleaved at the cell membrane, leaving the intracellular portion free to translocate to the nucleus and induce transcription.

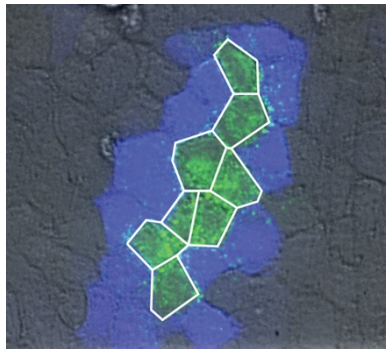
To generate synthetic Notch receptors, Lim and his team replaced the extracellular and intracellular domains with different sensor and response domains while maintaining the transmembrane domain (Morsut *et al.*, 2016). "We can put quite a variety of different things on the extracellular domain and get them to signal," explains Lim. Two decades ago, it was shown that the intracellular domain can also be exchanged. This modularity is possible because the mechanism of Notch activation involves mechanical forces. "Because it is a mechanical system it turns out that you can actually replace both the extracellular and the intracellular [domain of Notch] and therefore make a new class of receptors. You change what is detected and very flexibly can also change what the response is," says Lim.

The researchers created synthetic Notch (synNotch) receptors that could sense GFP or the tumor antigen CD19. On the response side, they used Gal4 or tTA transcriptional activators or a transcriptional repressor with a KRAB domain. Because Notch functions without any signaling intermediates, there is no cross-talk between different synthetic Notch receptors, and they can be used in a combinatorial manner. For example, two different synthetic Notch receptors can be combined to create an AND-gate through a split transcription factor that is reconstituted when both receptors are activated. "The key point is having the synNotch receptors control the functional release or production of a component that needs another component to work," says Lim.

The researchers demonstrated that they could use the synthetic Notch receptors individually, in combination or sequentially to change cell fate or to pattern fields of cells. Although such applications show the value of the synthetic Notch receptors as tools for basic research, they also have potential in translational applications. T cells have been engineered to kill cancer cells by expressing chimeric antigen receptors (CARs), but it is difficult to pinpoint single, cancer-specific antigens. Synthetic Notch receptors can increase the specificity of therapeutic T cells by making T cell activation dependent on two antigens (Roybal *et al.*, 2016).

In a proof-of-principle application, the researchers generated T cells that were active only in the presence of CD19 and GFP. They achieved this specificity through an AND-gate circuit in which GFP activated a synthetic Notch receptor, leading to the expression of a CD19-specific CAR and making the cells responsive to CD19.

The researchers showed in mice that the dual-receptor T cells killed tumor cells that expressed both antigens but spared cells that expressed only one or the other antigen. This specificity might seem surprising considering that after induction of CAR expression, circulating cells might encounter CD19-expressing cells that lack GFP. But



Synthetic patterning: GFP-expressing cells induce BFP production in cells harboring a GFP-specific synthetic Notch receptor. Adapted from Figure 4 in *Cell*, **164**, Morsut, L. *et al.*, Engineering customized cell sensing and response behaviors using synthetic notch receptors, 780–791, Copyright 2016, with permission from Elsevier.

"the lifetime of the primed state, we believe, is short relative to the time of redistribution of the cells and the actual tumor killing," explains Lim. CAR expression decays in about eight hours, whereas T cell activation and activation of the proliferative response take about twelve hours. "They are primed long enough, but on the timescale of what needs to happen to really clear a tumor, it's relatively short," says Lim.

Given the promising demonstration of dual-receptor therapeutic T cells, Lim is interested in translational applications of synthetic Notch receptors, especially disease applications that require local and precise recognition of tissues and cells. But he also thinks that the synthetic Notch platform is "a great research tool for exploring many other questions about how cells communicate with one another to achieve more complex behavior."

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RESEARCH PAPERS

Morsut, L. *et al.* Engineering customized cell sensing and response behaviors using synthetic Notch receptors. *Cell* **164**, 780–791 (2016).

Roybal, K.T. *et al.* Precision tumor recognition by T cells with combinatorial antigen-sensing circuits. *Cell* **164**, 770–779 (2016).