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Spotlight

Synthetic Notch receptors and their applications to study cell-cell contacts *in vivo*

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Physical cell-cell interaction is a fundamental mechanism that controls the development and physiology of multicellular organisms. However, methods to study cell contact within complex tissues are limited. In a recent issue of *Science*, Zhang et al. developed synthetic Notch (synNotch)-based tools that can monitor and trace cell-cell contacts in mammals.

Many biological processes in metazoans, including embryonic development, neurotransmission, immune response, and tumorigenesis, depend on direct cell-cell interactions. Detecting and controlling cell-cell contact *in vivo* not only helps reveal how multicellular organisms are functionally organized but also provides techniques for manipulating cell-cell interactions for various applications, such as cancer immunotherapy and tissue engineering. Currently, many tools have been developed to monitor cell-cell contacts *in vivo*, including protein complementation and proximity labeling.[1] However, these approaches are limited in certain aspects; cell-cell contacts must be maintained during detection, only provide snapshots of the current contact status, and do not allow future control of the labeled cells. In contrast, synthetic receptors with a transcriptional readout are more versatile, with the potential to manipulate, either transiently or permanently, the cells that have been in contact. Among existing synthetic receptors, synthetic Notch (synNotch) is currently the most widely used.

The Notch receptor is an evolutionarily conserved membrane protein in metazoan well characterized for its role in contact-dependent cell-fate determination. After binding to its ligand, Notch is activated through regulated intramembrane proteolysis to the release of its intracellular domain as a transcriptional activator. The synNotch receptor was developed initially to test the long-held hypothesis that Notch is activated by mechanical forces.[2] In synNotch, the native Notch ligand-binding domain and intracellular domain are replaced with artificial ligand-binding module and transcriptional factor, respectively (Figure 1A). Therefore, synNotch cannot be activated by endogenous ligands nor trigger its native transcriptional targets. The binding of an artificial ligand triggers cleavage of synNotch and releases the transcriptional factor, which activates downstream gene expression (Figure 1B). Applications of synNotch as a bio-orthogonal tool were tested in both mammalian and *Drosophila* cells. In mammalian cells, synNotch was used as a chimeric antigen receptor for T cell immunotherapy.[3] In *Drosophila*, synNotch was used to detect cell-cell contacts in the nervous system, as well as monitor and manipulate interactions between different cells during development.[4–6] A recent study by Zhang et al. builds on and greatly extends the synNotch system to detect and record *in vivo* intercellular contacts within complex mammalian tissues.[7]

Zhang et al. generated a collection of transgenic mice carrying different synNotch systems. The authors first created "genetic labeling of cell-cell contact" (gLCCC) to detect current cell-cell contacts. In gLCCC, formation of cell-cell contacts facilitates binding between the artificial ligand and synNotch receptor, which triggers synNotch cleavage, release of the intracellular transcriptional factor, and hence activation of reporter gene expression (Figure 1C). Next, they generated "genetic tracing of cell-cell contact" (gTCCC) to record the long-term cell contact history. In gTCCC, Cre DNA recombinase is under the control of synNotch. Activation of synNotch triggers Cre-mediated recombination to excise a STOP cassette and activate target gene expression. Thus, cells that form contact and their progeny are permanently labeled (Figure 1D). The authors also combined the gLCCC and gTCCC systems to label cells currently in contact and cells with a contact history. Moreover, Zhang et al. applied synNotch to label endothelial cells that contact transplanted ligand-expressing tumors and used flow cytometry and RNA sequencing (RNA-seq) to analyze the effect of tumors on the associated endothelium. They made several interesting observations, including the developmental origin of the liver vasculature from the early heart and the migration of tumor-associated endothelial cells into surrounding tissues. In the future, these synNotch mice should facilitate studies of various biological processes, such as stem cell niche interactions, lymphocyte homing, tumor metastasis, and neuronal connectivity. In addition, this synNotch can be used to manipulate interacting cells, which may facilitate mechanistic studies and even have therapeutic applications, such as use of lymphocytes with engineered responses to specific target cells and stem cells that release cytokines or differentiate into functional types after contacting target tissues.

Despite the promises of this synNotch system, several improvements could be made. First, whether the artificial ligand-receptor like synNotch causes unwanted cell-cell adhesion or other undesirable

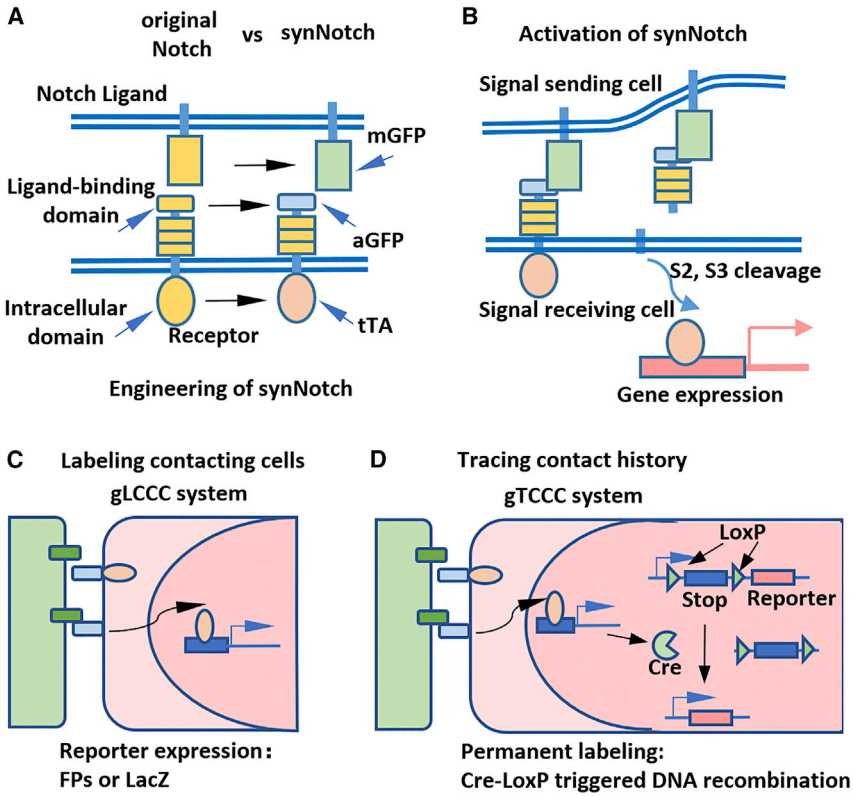

Figure 1. Detection and tracing of cell-cell contact using synNotch

(A) In the study of Zhang et al., a membrane-tethered GFP (mGFP) and an anti-GFP nanobody (aGFP) were used as the artificial binding pair, and the tetracycline transactivator (rTA) was used for binary gene expression.

(B) Activation of synNotch releases the membrane-tethered transcriptional factor and triggers downstream gene expression.

(C) gLCCC system: current cell-cell contact is detected by the expression of a fluorescent protein or lacZ.

(D) gTCCC system: permanent labeling of the contacted cells and their progeny is achieved by expressing a DNA recombinase Cre, which triggers DNA rearrangement and reporter gene expression.

effects remains to be thoroughly tested—although several previous studies have suggested that the mechanical changes induced by synNotch may be insignificant. For example, the force required to trigger Notch cleavage and activation is comparatively low, just 3–5 pN, which is about one-tenth of the strength of classic cadherin and integrin.[2] Further, in flies, ubiquitous expression of synNotch and its ligand in different tissues, including migratory cells that are sensitive to cell-cell adhesion changes, has not caused any significant defects.[5] Nevertheless, to alleviate this potential concern, Zhang et al. adapted a synNotch version with a low-affinity anti-GFP nanobody with a Kd of 50nM, which is about one-tenth of the affinity between integrin and its RGD substrate.

Second, the synNotch receptor may be affected by endogenous signaling, as ligand-independent activation occurs in some cells.[8] Such activation of synNotch has also been reported in *Drosophila* and mammalian cells.[5,9] With the development of synNotch tools in mice, it will be important to test whether ligand-independent activation of synNotch exists in some cells or conditions. Interestingly, changing the number of extracellular EGF repeats or adding a short native sequence adjacent to the transmembrane domain of Notch alters this ligand-independent background in both fly and mammalian cells.[2,5,9] It may be possible to develop enhanced synNotch with reduced background and increased sensitivity.

Third, unlike other receptors, like GPCRs and RTKs, which use enzyme cascades to amplify the original signal, synNotch has no signal amplification mechanism before transcriptional activation, which may limit its use to detect transient cell-cell connections or cells sharing small contact surfaces like synapses or cytonemes. One option to increase sensitivity would be to target the ligand or receptor to specific subcellular structures to increase its local concentration. For example, while a regular membrane-tethered GFP ligand failed to activate synNotch in neurons through synaptic connection, targeting it specifically to the synapse achieved *trans*-synaptic labeling of synNotch.[5,6] Alternatively, adding a signal amplification circuit, such as an enzyme-cascade or transcriptional positive-feedback loop, may also enhance the sensitivity of synNotch.

In summary, the synNotch-based tools generated by Zhang et al. in mice are likely to be used widely to study biological processes such as embryogenesis, tumorigenesis, and tissue regeneration. In addition, as synNotch-labeled cells can be isolated, the approach can be further combined with different omics techniques, such as single-cell RNA-seq (scRNA-seq) or mass spectrometry (MS)-based proteomics, to characterize the molecular consequences of intercellular interactions.

DECLARATION OF INTERESTS

N.P. is on the advisory board of *Developmental Cell*.

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