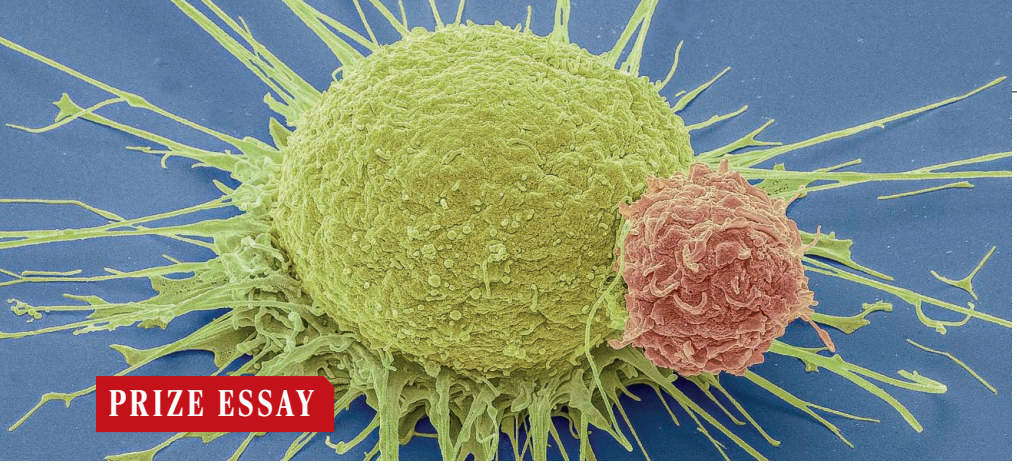

PRIZE ESSAY

REGENERATIVE MEDICINE

Refining cell therapy

Synthetic Notch receptors expand the therapeutic potential of engineered T cells

By **Kole T. Roybal**

We are in the midst of a major shift in how we think about treating disease and the regeneration of damaged tissues. Although small-molecule and protein therapeutics are the dominant forms of treatment today, we are now at the point where we can engineer our own body's cells to detect and treat disease. Using the cutting-edge tools of synthetic biology, we may one day be able to build smart therapeutic cells that reside in the body for life, poised to respond to diseases that would otherwise thwart our natural immune system.

My colleagues and I envision cell therapies that act as microscopic "physicians," capable of detecting, diagnosing, and directly eradicating disease via a multifaceted mechanism that is difficult to resist and circumvent. This therapeutic vision contrasts with more traditional drug therapies, which often require chronic administration and generally target individual disease mechanisms that are easily bypassed, resulting in disease recurrence.

T CELL THERAPY: CURRENT CHALLENGES

The promise of engineered cell therapy is beginning to be realized with the remarkable success of T cell therapies for cancer. T cells engineered to express chimeric antigen receptors (CARs)—synthetic receptors consisting of a tumor-specific extracellular antibody fragment fused to the signaling chain from the native T cell receptor—can target and eliminate difficult-to-treat B cell malignancies that have few therapeutic alternatives.

While exciting, these therapies suffer from some challenges. The inability to control CAR T cells, off-target tissue damage, and unchecked inflammation has led to severe adverse effects in some patients. Treatment of solid tumors with T cell therapies has been largely ineffective due to problems with infiltration and immunosuppressive tumor microenvironments. With these common pitfalls of T cell therapies in mind, I sought to develop a new class of synthetic receptors that would allow cells to initiate more customized, controlled, and localized therapeutic activity.

Although CARs can retarget T cells to tumors, in doing so, they elicit the full T cell response that includes elements that are both

therapeutic and toxic. To develop a new class of receptors for more precise control over cellular behavior, I teamed up with a fellow postdoctoral researcher, Leonardo Morsut, and looked to the Notch receptor, a classic receptor involved in development and a myriad of other biological processes.

CREATING SYNTHETIC NOTCH RECEPTORS

The Notch receptor is mechanistically direct, in that ligand binding leads to proteolytic cleavage of the cytoplasmic domain, which is then transported into the nucleus, where it acts as a transcriptional regulator. Notch receptors do not initiate the activation of complex kinase cascades like most other receptor families. Thus, we reasoned that Notch could be a perfect platform on which to build custom environmental sensors that detect disease- or tissue-specific cues and initiate a more streamlined custom therapy.

By engineering chimeric versions of the Notch receptor, we found that both the extracellular ligand-binding domain and the intracellular domain of Notch can be readily exchanged, such that synthetic versions of the receptor can be built with a user-defined ligand-binding module (e.g., a single-chain variable fragment or nanobody to a tissue- or disease-related antigen) and an orthogonal intracellular transcriptional regulator (to control a custom genetic program) (*1*).

Synthetic Notch (synNotch) receptors have proven to be a versatile platform for cellular environmental sensing coupled to precise gene regulation. The cellular machinery required for Notch activation is ubiquitously expressed, meaning that we can engineer a spectrum of cell types with therapeutic synNotch receptor circuits. For example, a stem cell could be engineered with a synNotch receptor circuit that senses damaged tissue and coordinates tissue repair by locally secreting growth factors.

A LA CARTE CELL THERAPY

Many of the therapeutics that aid in cancer clearance are genetically encodable (e.g., cytokines, antibodies, and toxins) and could benefit from more targeted delivery because they are ineffective or toxic when administered systemically. We have established that synNotch receptor circuits in T cells can drive a la carte secretion of cytokines, biased T cell differentiation, and local delivery of non-native therapeutic payloads, such as antibodies, in response to tumor antigens (*2*).

SynNotch receptors are also able to improve the specificity of engineered T cells for tumors when they are combined with CARs (*3*). T cells engineered with synNotch receptors and CARs require a combination of antigens to activate instead of a single tumor antigen. These dual-antigen-targeted synNotch/CAR T cells initially only express the synNotch receptor targeted to a primary tumor antigen. Upon recognition of that antigen, the receptor drives the expression of a CAR to a second tumor antigen. The control of CAR expression by a tumor-targeted synNotch receptor effectively confines T cell activity to the tumor.

Given that single, highly specific tumor antigens are rare, combinatorial antigen sensing by cells is a powerful approach to enhance the discrimination of tumors from off-target tissue. We have recently identified clinically relevant combinatorial tumor antigen signatures and have built synNotch/CAR

T lymphocyte cells (pink) attach to a prostate cancer cell in this electron micrograph.

PHOTO: STEVE GSCHMEISSNER/SCIENCE SOURCE

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GRAND PRIZE WINNER

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Kole Roybal is an assistant professor in the Department of Microbiology and Immunology at the University of California, San Francisco, a member of the Parker Institute for Cancer Immunotherapy, and a Chan Zuckerberg Biohub Investigator. His laboratory harnesses the tools of synthetic and chemical biology to engineer the immune cell therapies for cancer and autoimmunity of the future. He received his doctorate from the University of Texas Southwestern Medical Center at Dallas. There he studied the fundamental cellular and biochemical mechanisms required for immune cell activation and clearance of infections. While a Jane Coffin Childs Postdoctoral Fellow in Wendell Lim's laboratory at UCSF, he developed a new class of synthetic receptors, which provide unprecedented customization of therapeutic cells for treatment of a broad range of diseases. **www.sciencemag.org/content/359/6380/1112**

FINALIST

Shruti Naik

Shruti Naik received her B.S. in cell and molecular biology from the University of Maryland and her Ph.D. in immunology from the University of Pennsylvania–National Institutes of Health Graduate Partnership Program. During her graduate training, she discovered that normal bacteria living on our skin educate the immune system and help protect us from harmful pathogens, opening the door for microbiota-based therapies in the skin. She is currently at Rockefeller University, where she is studying the interactions between immune cells and stem cells in an effort to develop stem cell–based therapies for inflammatory disorders. She is also a strong advocate for women in science.
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FINALIST

Fotios Sampaziotis

Fotios Sampaziotis graduated from the University of Athens in Greece with a degree in medicine. He obtained a Ph.D. in stem cell biology from the University of Cambridge. During his doctoral research, he pioneered the use of bile duct organoids to model diseases of the biliary system, test multiple drugs, and identify new therapeutic agents. Currently, Fotios continues his research at the interface between basic science and clinical medicine as a clinical lecturer in hepatology at the University of Cambridge with clinical commitments in Addenbrooke's Hospital. His scientific work focuses on combining organoids, bioengineering, and animal studies to regenerate damaged bile ducts in the liver as an alternative therapy to liver transplantation. **www.sciencemag.org/content/359/6380/1113.2**

FINALIST

Will McLean

As an undergraduate, Will McLean studied biology at Tufts University before going on to attain a Ph.D. at the Massachusetts Institute of Technology within the Harvard-MIT Division of Health Sciences and Technology. While at MIT, his doctoral research elucidated the distinct progenitor cell types that exist within the inner ear and their capacity to form sensory cells and neural cell types. As a postdoctoral researcher at Harvard Medical School, he investigated manipulation of signaling pathways to enable otherwise senescent progenitor cells of the cochlea to divide and form new sensory cells. He is currently vice president of biology and regenerative medicine at Frequency Therapeutics. Frequency is currently using McLean's insights to develop a drug to treat hearing loss by regenerating lost sensory cells.
www.sciencemag.org/content/359/6380/1113.3

circuits capable of recognizing them, with the hope of rapidly moving this new cell therapy to the clinic.

SynNotch receptors provide unprecedented control and customization of cell activity and have far-reaching implications for cancer, autoimmunity, and regenerative medicine. In the future, we hope to use synNotch T cells to remodel immunosuppressive tumor microenvironments and improve the efficacy of T cell therapies for solid tumors. We also hope to go beyond the treatment of cancer and develop synNotch engineered T cells that can detect and suppress autoinflammatory disease.

REFERENCES

1. L. Morsut *et al.*, *Cell* **164**, 780 (2016).
2. K. T. Roybal *et al.*, *Cell* **167**, 419 (2016).
3. K. T. Roybal *et al.*, *Cell* **164**, 770 (2016).

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