Genetic Control of T-Cell Proliferation with Synthetic RNA Regulatory Systems

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Abstract

Adoptive T-cell therapy, or the use of autologous T cells to seek and destroy diseased cells, is a promising treatment option for opportunistic diseases, virus-associated malignancies, and cancers. However, the safety and efficacy of adoptive T-cell therapy depend, in part, on the ability to sustain and tightly regulate the proliferation of transferred T cells *in vivo*. The emerging field of synthetic biology provides powerful conceptual and technological tools for the construction of regulatory systems that can interface with and reprogram complex biological processes such as cell growth. Here, we present the development of RNA-based regulatory systems that can control T-cell proliferation in a ligand-dependent manner, and examine the construction of integrated control systems capable of fine-tuned programming of cellular behavior.

We systematically investigate the translation of ribozyme-based regulatory devices from yeast to mammalian cells and identify design parameters critical to the portability of regulatory devices across host organisms. We report the construction of ligand-responsive ribozyme switch systems capable of modulating the transgenic expression of growth-stimulatory cytokines in mammalian lymphocytes. We demonstrate the ability of ribozyme switch systems to regulate T-cell proliferation in primary human central memory T cells and in animal models. We further develop ligand-responsive, miRNA-based devices to regulate the endogenous expression of cytokine receptor chains and the functional output of cytokine signaling pathways, highlighting the ability to construct integrated T-cell proliferation control systems employing various regulatory mechanisms to modulate multiple components in relevant signaling pathways. Finally, we describe efforts in the generation of novel RNA aptamers to clinically suitable

molecules, which can serve as the molecular inputs for ligand-responsive, RNA-based control systems in therapeutic applications.

The regulatory systems developed in this work are designed to be modular and transportable across host organisms and application contexts, thus providing a template for future designs in RNA-based genetic regulation. This work demonstrates the capability of RNA-based regulatory systems to advance next-generation treatment options for critical diseases, and highlights the potential of synthetic biological systems to achieve novel and practical functions in diverse applications.

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