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Building with intent: Technologies and principles for engineering mammalian cell-based therapies to sense and respond

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Abstract

The engineering of cells as programmable devices has enabled therapeutic strategies that could not otherwise be achieved. Such strategies include recapitulating and enhancing native cellular functions and composing novel functions. These novel functions may be composed using both natural and engineered biological components, with the latter exemplified by the development of synthetic receptor and signal transduction systems. Recent advances in implementing these approaches include the treatment of cancer, where the most clinical progress has been made to date, and the treatment of diabetes. Principles for engineering cell-based therapies that are safe and effective are increasingly needed and beginning to emerge, and will be essential in the development of this new class of therapeutics.

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Abbreviations

CAR, Chimeric antigen receptor; GLP-1, Glucagon-like peptide-1; HEK, Human embryonic kidney; IL, Interleukin; iPSC, Induced pluripotent stem cell; MESA, Modular extracellular sensor architecture; TCR, T cell receptor; TF, Transcription factor; TNF, Tumor necrosis factor; VEGF, Vascular endothelial growth factor.

Introduction

Engineered cell-based therapies are a powerful and rapidly advancing frontier in medicine. Cells can perform functions that are currently inaccessible by pharmacological means, including directed trafficking within the body, sustained production of therapeutics *in situ*, and performance of complex tasks such as cell-mediated killing. In this review, we focus on a promising facet of this approach—the potential to engineer cells to carry out defined sense-and-respond behaviors in which the therapeutic interfaces with the host environment. As discussed below, the promise of this approach has been demonstrated by recent successes in the treatment of certain cancers. Thus, the need for tools and principles for designing and engineering cells to perform increasingly sophisticated and robust functions represents a pressing challenge in biomedical engineering and in mammalian synthetic biology.

The expanding suite of genetic parts and their applications for engineered cell therapies have been extensively reviewed elsewhere [1–3], and thus here we endeavor to provide a conceptual framework for considering and ultimately designing new therapies. First, we survey recent progress in the field, focusing on examples that illustrate distinct modes by which cell therapies can be engineered to sense and modulate host physiology. We highlight how each application utilizes native functions, novel functions, or both. Second, we draw upon experience gained from these examples and others to summarize current understanding of principles and challenges for engineering cell-based therapies that may ultimately achieve clinical benefits in diverse applications.

Potentiating, controlling, and recapitulating native functional modalities

Enhancing the T cell response to cancer

A forefront example of modifying cells to potentiate and enhance a native functionality is engineering T cells for cancer therapy [2,4]. The chimeric antigen receptor

(CAR) is an engineered T cell receptor (TCR) that confers programmable antigen specificity and enhanced activation and persistence to T cells upon antigen recognition. CAR T cell therapies have improved survival in an increasing number of cancer clinical trials [5]. The modular CAR structure renders these receptors readily modifiable to recognize tumor antigens, modulate signaling, and increasingly implement sophisticated refinements upon the control of T cell activation to address specific clinically-observed challenges; these topics are reviewed extensively elsewhere [2]. A distinct recent approach utilized native transcriptional regulation to address challenges associated with CAR therapies: using Cas9 DNA editing to express the CAR from the native TCR locus minimized antigen-independent signaling and promoted recovery to basal levels after antigen-induced receptor internalization, thereby reducing the problem of T cell exhaustion [6]. The success of CAR T cells in clinical trials [5], recent FDA approval of two CAR T cell therapies for acute lymphoblastic leukemia (tisagenlecleucel/Kymriah) and large B-cell lymphoma (axicabtagene ciloleucel/Yescarta) [7,8], and substantial commercialization of CAR technologies have all paved the way for cell-based therapies with functionalities beyond cell-mediated killing.

Controlling blood sugar in diabetes

Diabetes is a disorder of blood sugar regulation that results from either a deficiency of insulin-producing beta cells or an insensitivity to insulin. The ultimate cure for type 1 diabetes is replacement of beta cell function, but pancreas or islet cell transplant is still limited to select patients [9]. Transplantation of differentiated beta cells has been investigated as an alternative to transplantation of the pancreas or islet cells, however obtaining physiologic glucose-sensing and insulin production remains a challenge [10]. One approach that could address the need for beta cells is to differentiate human induced pluripotent stem cells (iPSCs) into beta-like cells. Toward this end, a synthetic lineage-control gene expression network was developed to induce the sequential expression of multiple pancreatic transcription factors (TFs) under external control of vanillic acid provided in the medium. This strategy generated iPSCs that released insulin in a glucose-responsive manner, matching the necessary human physiological input/output ranges [11].

An alternative strategy to transplanting beta cells is expression of the components required for glucose-responsive insulin secretion in non-beta cells. Early work included constitutive insulin expression in a hepatic cell line [12] and inducible insulin expression using nanoparticle-mediated local heating to induce a calcium-responsive promoter via a heat-responsive ion channel [13]. More recently, genetic circuits have been developed that place the expression of a glucagon-like peptide 1 (GLP-1) analog, which enhances insulin secretion, under control of blue light [14] or oleanolic acid, which may be orally administered [15]. Notably, these circuits rely upon external or open-loop control to regulate blood sugar, rather than the closed-loop control achieved by natural beta cells. However, closed-loop glycemic control was recently demonstrated by engineering non-beta HEK293T cells to produce insulin in response to glucose [16]. Microencapsulation and implantation of these cells, which precluded cellular infiltration and exfiltration from the capsule while allowing diffusive transport, restored diabetic mice to normoglycemia. Closed-loop control has also been implemented using a combination of biological and electronic technologies. Insulin production was coupled to LED implant-generated far-red light under the control of a computer (or smartphone) that uses a glucometer to achieve real-time feedback control. If desired, this setup allows for human intervention as a safety precaution [17]. In a novel strategy for the treatment of type 2 diabetes, a signaling pathway was engineered to produce an adiponectin derivative, a hormone that increases insulin sensitivity, in response to hyperinsulinemia [18]. These studies show how synthetic recapitulation and augmentation of natural functions can address specific clinical needs.

Building new therapeutic functional modalities

A unique prospect for engineered cell therapies is the potential to go beyond enhancing and controlling native functions to construct novel therapeutic modalities that cells do not otherwise exhibit. In this section, we first discuss recent progress in building novel functions by repurposing natural biological parts. We then consider recent technological advances that enable one to direct how a cell senses features of its environment in a customizable fashion using synthetic receptors.

Engineering novel cellular functions by repurposing natural parts

Many diseases may be viewed as disorders of homeostasis, with therapeutic intervention aimed at restoring a natural balance. Among these, chronic inflammatory diseases provide examples in which engineered sense-and-respond has been applied by repurposing natural parts to build cellular “devices” that counter the pathological inflammation. Psoriasis, arthritis, and inflammatory bowel disease involve dysregulated immune responses and increased expression of pro-inflammatory cytokines. Since systemic ablation of pro-inflammatory cytokines or their action can result in profound immunosuppression, a more desirable therapeutic modality is to utilize feedback control. A recent application of this approach to psoriasis leveraged native receptors and intracellular signaling cascades to sense the pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin (IL-22). A downstream gene circuit was used to implement an AND gate for producing the anti-