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Synthetic mammalian gene circuits for biomedical applications

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Synthetic biology is the science of reassembling cataloged and standardized biological items in a systematic and rational manner to create and engineer functional biological designer devices, systems and organisms with novel and useful, preferably therapeutic functions. Synthetic biology has significantly advanced the design of complex genetic networks that can reprogram metabolic activities in mammalian cells and provide novel therapeutic strategies for future gene-based and cell-based therapies. Synthetic biology-inspired therapeutic strategies provide new opportunities for improving human health in the 21st century. This review covers the most recent synthetic mammalian circuits designed for therapy of diseases such as metabolic disorders, cancer, and immune disorders. We conclude by discussing current challenges and future perspectives for biomedical applications of synthetic mammalian gene networks.

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Introduction

Synthetic biology is the science of designing and creating novel functional devices, systems, and new organisms by applying engineering principles [1–3]. In the early stages of synthetic biology, many synthetic devices were designed and constructed in prokaryotes or lower eukaryotes, including toggle switches [4], oscillators [5–7], timers [8], counters [9], clocks [10], pattern detectors [11], band-pass filters [12], and intercellular communication systems [13,14]. Pioneering experiments later confirmed the therapeutic possibilities of synthetic biology. These experiments included designing bacteriophage to switch off the bacterial SOS DNA repair system [15], engineering bacteria or viruses for cancer-targeting therapy [16–21], engineering *Escherichia coli* to prevent cholera infection [22], and engineering yeast cells to produce the precursor of the antimalarial drug artemisinic acid [23]. Although synthetic devices can easily be engineered and programmed in prokaryotic systems, the clinical application of prokaryotic synthetic circuits is limited. Recent studies have therefore focused on designing heterologous transcription-control systems in mammalian cells. This research opens a door for developing new therapeutic strategies, especially gene-based and cell-based therapies, to treat human diseases [24,25•,26–28] (Figure 1). This review focuses on the latest in mammalian synthetic biology, including designing state-of-the-art, synthetic gene networks and developing prototype treatment strategies.

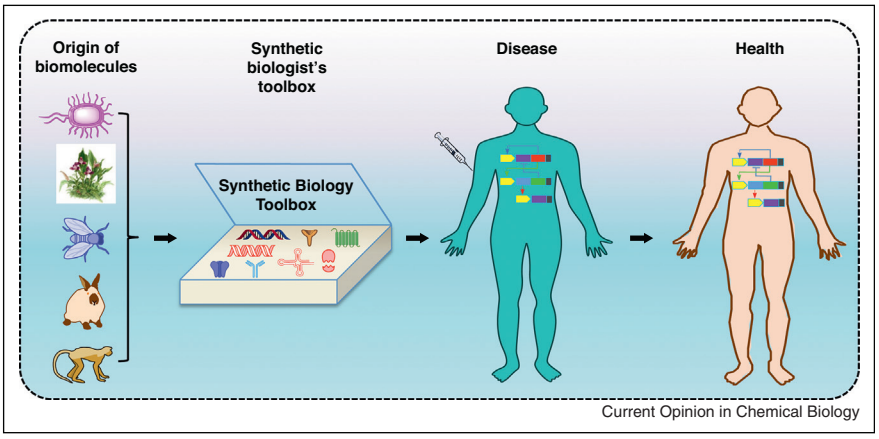
Synthetic biology therapeutic strategies for metabolic disorders

Type 2 diabetes is a metabolic disorder characterized by high blood glucose as a result of insulin resistance or relative insulin deficiency [29]. It is common both in developed and developing countries and approximately 6 percent of the world's population is affected by it. Traditional therapeutic strategies include a strict diet and increased exercise, daily insulin injections, or medication before every meal. These strategies combine high cost with low patient convenience.

Two highly promising, synthetic biology-based therapeutic strategies for treating diabetes were recently demonstrated. The first example is the construction of an optogenetic device to control blood glucose homeostasis [30••]. Using light as a traceless, molecule-free, input signal to trigger transgene expression in living organisms is becoming popular [31–35]. Ye *et al.* engineered expression of shGLP-1, a hormone that can restore blood glucose homeostasis in type 2 diabetic mice, to be under the control of blue light. Melanopsin, a blue-light sensor protein, is ectopically expressed in HEK-293 cells, and when triggered by the presence of blue light, activates the nuclear factor of activated T cells (NFAT) by phosphorylation via an intracellular signaling cascade. This cascade results in HEK-293 cells expressing shGLP-1, which has been placed under the NFAT-controlled promoter. These engineered cells were then encapsulated and implanted into a mouse model of human type 2 diabetes. Mice illuminated by blue light showed enhanced blood-glucose homeostasis (Figure 2a). Another study later confirmed that diabetes treatment by light-triggered GLP-1 expression was possible [36].

Stanley *et al.* [37••] developed an insulin expression system under the control of radio waves to restore glucose

Figure 1



Synthetic gene circuits designed for treating human disease. Biologists capitalize on natural biomolecules from various organisms (including microbes, plants, and mammals), which are well understood and have been biochemically studied. The synthetic biology toolbox consists of those well-studied, high-value biomolecules that consist of functional DNA, RNA, and proteins. By applying engineering principles, synthetic biologists reassemble and standardize these basic toolbox components in a rational way to create and engineer functional therapeutic gene networks. These networks are then uploaded into encapsulated cell implants, which are placed into the body and regulated to produce valuable therapeutic biomolecules. These biomolecules treat the disease, and bring the patient back to health.

homeostasis in diabetic mice. In this system, a temperature-sensitive channel, TRPV1, is antibody-coated with oxide nanoparticles, and therefore triggers calcium influx when heated by radio waves. This influx of calcium stimulates both the expression and release of insulin, from a bioengineered insulin construct driven by a calcium-sensitive promoter. When the mice harboring tumor xenografts expressing insulin are exposed to radio waves, the system is activated to express and release insulin from the tumors and lower blood glucose (Figure 2b).

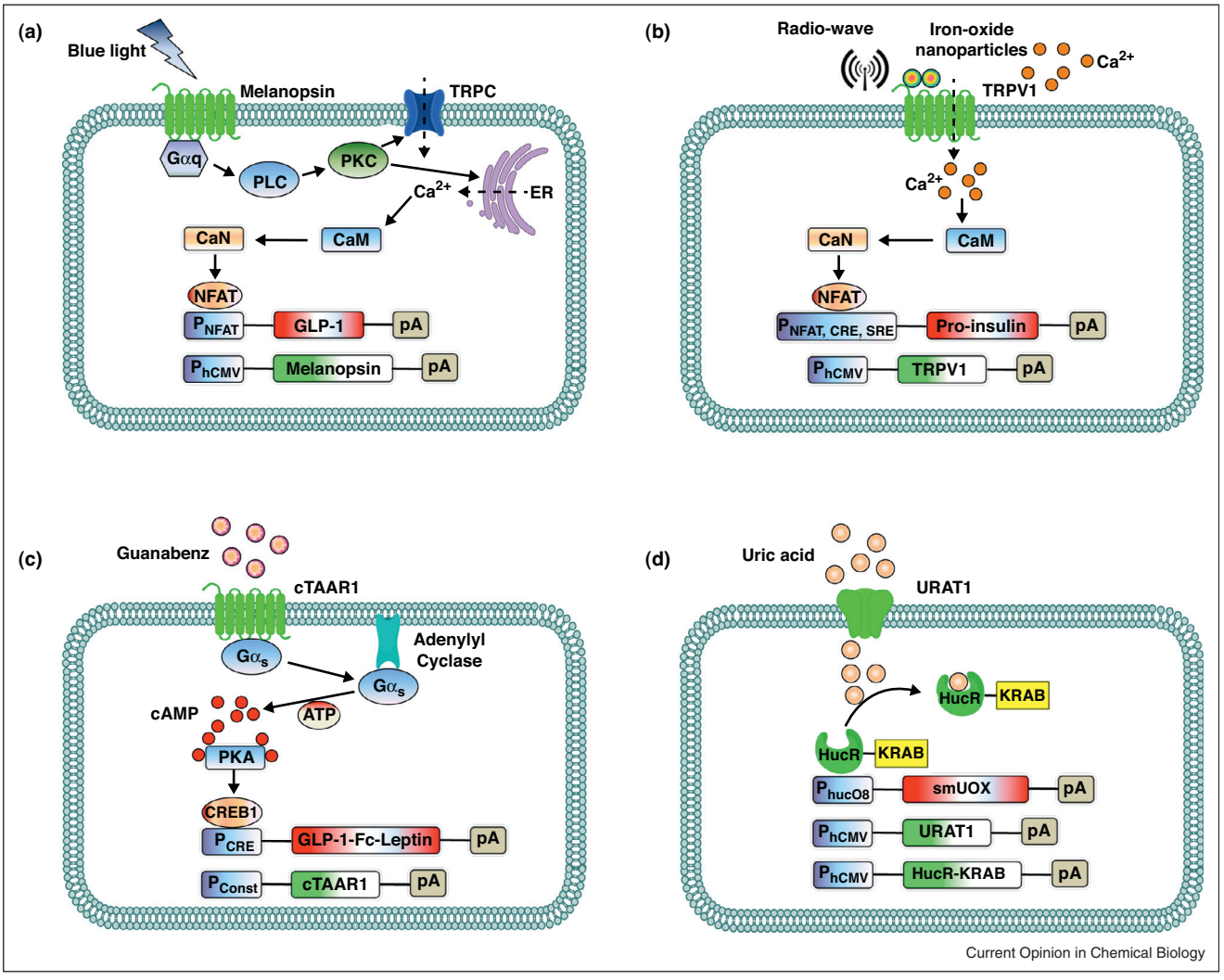
Metabolic syndrome, a prime 21st-century epidemic, is a combination of disorders and risk factors, including hypertension, hyperglycemia and dyslipidemia, all of which collectively increase the risk of cardiovascular disease [38,39]. The traditional therapeutic strategy is to identify and treat each risk factor separately [40]. However, this single-risk-factor treatment strategy can cause polypharmacy which represents a lifestyle disincentive for patients and may accumulate side effects [40]. Recently, a multifunctional synthetic circuit for simultaneously treating multiple risk factors of metabolic syndrome was developed [41••]. In this circuit, the signal transduction of the chimeric trace-amine-associated receptor 1 (cTAAR1), triggered by the antihypertensive drug guanabenz (Wytensin®), is functionally rewired to use cAMP and cAMP-dependent PKA. This then activates the cAMP-response element binding protein (CREB1), which binds to the CREB1-specific promoter driving the therapeutic peptide hormone GLP-1-Fc-Leptin. This system has been successfully engineered and implanted into mice developing metabolic syndrome symptoms and simultaneously attenuate hypertension, hyperglycemia, and dyslipidemia in mice (Figure 2c). This combination of classic and synthetic biology-based treatments may improve treatment success and provide new therapeutic strategies for multifactorial diseases.

A more advanced synthetic biology-derived prosthetic network would be able to sense and monitor host metabolic parameters and respond accordingly without further input. An example of this type of sensor-effector prosthetic network was designed to sense the uric acid concentrations in the blood, and control the expression of a urate oxidase (smUOX), which acts to restore urate homeostasis. This could be a treatment strategy for gout and tumor lysis syndrome [42] (Figure 2d). In this circuit, the smUOX expression level is controlled by the bacterial HucR repressor, which has been fused with the transcription-silencing domain KRAB (HucR-KRAB). Upon binding to the cognate operator site, HucR-KRAB inhibits smUOX production. In the presence of urate, the HucR-KRAB is released from the operator site, which initiates the expression of smUOX. Engineered cells containing this prosthetic uric-acid-responsive transcription network were implanted into acute hyperuricaemic mice. This network was able to dissolve uric-acid crystal deposits in the kidneys, proving that this concept works to ameliorate disease symptoms.

Synthetic logic circuits for cancer therapy

Cancer is a large group of diseases involving rapid, uncontrollable creation of abnormal cells that can invade nearby healthy tissues and spread to other organs. One of the key challenges for cancer therapy is to eliminate

Figure 2



Synthetic gene circuits designed for metabolic therapy. **(a)** Blue-light-triggered transcriptional control of blood-glucose homeostasis in mice. The heterologous expression of the G-protein-coupled receptor (GPCR) melanopsin is activated by blue-light illumination, and sequentially turns on a Gαq-type G protein, phospholipase C (PLC), and phosphokinase (PKC). This then triggers calcium influx both through transient receptor potential channels (TRPCs) and from the endoplasmic reticulum (ER). The calcium surge in the cytoplasm activates calmodulin (CaM) to become calcineurin (CaN), which in turn activates the nuclear factor of activated T cells (NFAT) by dephosphorylation. The dephosphorylated NFAT then translocates into the nucleus, where it binds to NFAT-specific promoters (P_{NFAT}) and drives glucacon-like peptide-1 (GLP-1) expression. The engineered cells containing this synthetic gene circuit were encapsulated and implanted into diabetic mice. When mice were illuminated by blue light, the synthetic gene circuit was activated and restored blood-glucose homeostasis in diabetic mice. **(b)** Radio-wave-triggered transcriptional control of blood-glucose homeostasis in mice. Iron-oxide nanoparticles bind to the 6 × His-tagged, transient-receptor-potential-cation channel subfamily V member 1 (TRPV1). Upon exposure to radio waves, local nanoparticles are heated, which activate the temperature-sensitive TRPV1 channel, and induce calcium influx. The calcium surge triggers downstream signaling pathways, such as calmodulin (CaM) activation to calcineurin (CaN), which in turn activates NFAT by dephosphorylation. The activated NFAT then binds to promoters that specifically recognize it (P_{NFAT}) and initiates the expression of a bio-engineered human insulin gene. The calcium-dependent signaling pathways can also stimulate the expression of pro-insulin via CRE (cyclic AMP response element) and SRE (serum responsive element). Mice with xenograft tumors containing this synthetic circuit demonstrated glucose homeostasis when treated by radio frequency. **(c)** Guanabenz-triggered designer circuit for treating metabolic syndrome. The heterologous expression of a G-protein-coupled chimeric trace-amine-associated receptor (cTAAR1) is activated by adding the antihypertensive drug guanabenz. This turns on a $G\alpha_s$-type G protein, which activates the membrane-bound adenylyl cyclase that converts ATP into cAMP. The cAMP surge in the cytoplasm is rewired to activate the cAMP-dependent phosphokinase A (PKA), which further activates the cAMP-response element-binding protein 1 (CREB1). The activated CREB1 binds to a specific promoter (P_{CRE}) and drives expression of the fusion protein GLP-1-Fc-Leptin. Mice with metabolic syndrome that are treated with this synthetic circuit demonstrated attenuated hypertension and hyperglycemia, as well as obesity and dyslipidemia. **(d)** Self-sufficient synthetic circuits restore blood-urate homeostasis in mice. A urate transporter URAT1 is heterologously expressed to enhance the intracellular urate level in engineered cells. Urate levels are detected by the uric-acid sensor KRAB-HucR, which can inhibit transcription of the secreted urate oxidase variant (smUOX) by binding to its operator ($hucO_8$). When urate concentration reaches pathological levels, the uric acid binds to KRAB-HucR, and KRAB-HucR is released from its operator and initiates the expression of smUOX. This expression can lower the blood-urate level by oxidizing urate to 5-hydroxyisourate.

cancer cells without damaging the healthy tissue surrounding them [43]. The most recent advances in cancer-targeting treatment include engineered tumor-targeting bacteria [19–21,44], oncolytic viruses [18,45], and synthetic, cancer-specific killer switches [46•,47••,48••].

Precise discrimination and killing of cancer cells could be achieved by a dual-promoter integrator, which consists of two native promoters only activated in the simultaneous presence of two transcription factors [46•] (Figure 3a). Constructs are made in which cancer-sensing promoters placed in cancerous cells, are engineered to activate either a fusion protein GAL4, which is a DNA-binding domain fused to the Coh2 domain, or the VP16 domain fused to the DocS domain. When both fusion proteins are expressed, in response to cancer-indicating signals, the Coh2 and DocS domains assemble into a chimeric transcription factor recognized by a synthetic promoter that then activates the expression of a pro-apoptotic drug, TK1, resulting in targeted cancer cell death. This dual-promoter circuit uses the principle of digital-like logic AND gate responses, which would improve targeting precision and efficacy in malignant cells.

In another study, a cancer-cell classifier was developed to sense HeLa-specific endogenous miRNA states [47••] (Figure 3b). The cell-type classifier can sense an expression-level set of up to six endogenous miRNAs by using a synthetic logic circuit. It can induce apoptosis only in the absence of miR-17, miR-21 and miR30a, and in the presence of miR-142, miR144, and miR146a. Upon monitoring endogenous miRNA levels, this designer circuit is able to kill specific cancers.

A different device to control cancer is a synthetic RNA control device, which is designed to be a programmable sensing-actuation device to enable autonomous control over cellular behavior [48••]. The synthetic RNA device can sense subunits of the nuclear factor kappa B or β-catenin (which are markers of cancer cells) and express a cancer prodrug. This device only acts as a cell-killing switch in the presence of endogenous cancer markers, again leaving the healthy tissue unharmed. Engineered mammalian cells performing logic calculations according to the presence or absence of specific disease-related biomarkers may have great potential for more precise cell-based therapies.

Synthesis of genetic circuits for immune-mediated therapy

T cells are a type of white blood cells essential to the mammalian immune system, especially the response to invasive pathogens. Human T cells have great value for synthetic biology platforms because they can be easily isolated from patients, genetically engineered, and then returned into patients to treat cancer or chronic infection. A synthetic RNA control device was designed to externally control T-cell proliferation in mice [49•]. This control used a synthetic ribozyme switch to provide programmable, drug-mediated regulation of cytokine expression. Another example is reprogramming T cells for tumor immunotherapy [50••]. Porter *et al.* reprogrammed autologous T cells expressing chimeric antigen receptors to recognize the B-cell antigen CD19 characteristic of chronic lymphocyte leukemia. Once the reprogrammed T cells were returned into patients, they were activated to proliferate and kill cancer cells (Figure 4a).

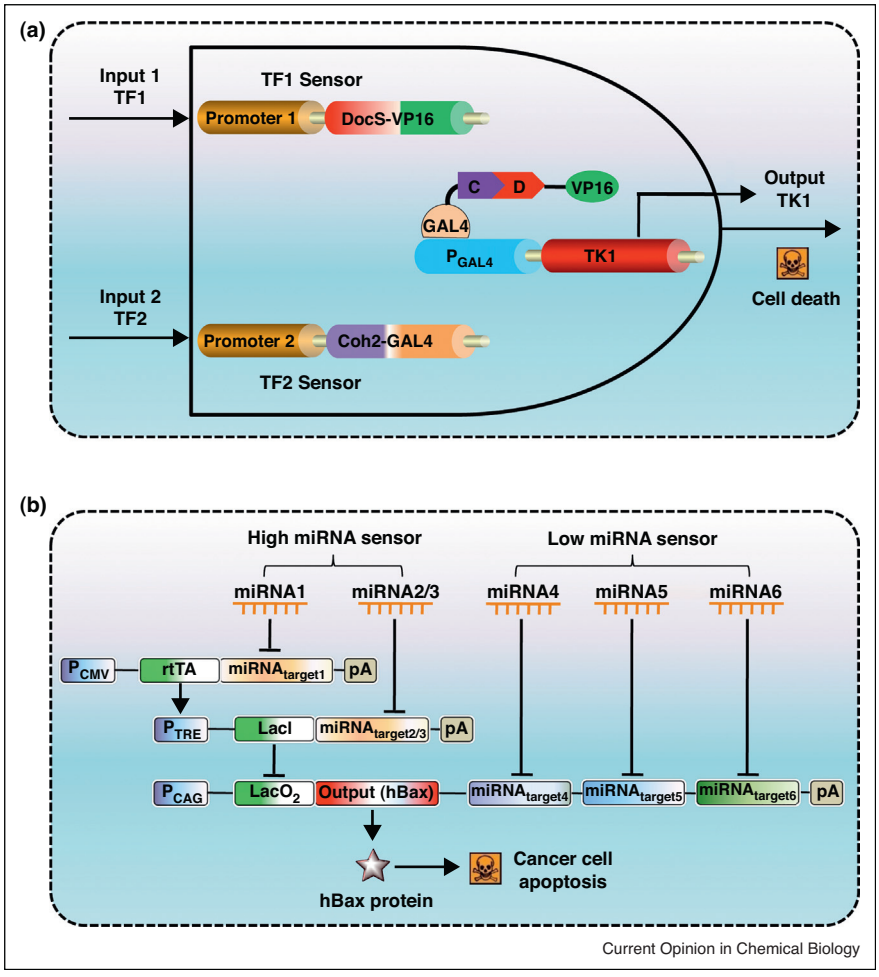
The therapeutic application of engineered T cells for autoimmune disorders has the risk of side effects [51]. In strategies employing T cells, both the specificity and amplitude of T-cell function need to be controlled. There is a need to balance therapeutic action and off-target toxicity, and to this end bacterial virulence proteins were recently explored as a toolkit to rewire kinase signaling pathways [52••] (Figure 4b). Bacterial effectors were used to create negative feedback loops to limit the maximum amplitude of T-cell function. Pause switches were also constructed by using bacterial effectors. These switches could allow external control of the timing and level of primary human CD^+ T-cell activation and cytokine release, in order to minimize the risk of a cytokine storm. These studies demonstrate the therapeutic applications of using bacterial pathogens as a toolkit to create feedback modulators or inducible pause switches, which will enable tuning of the T-cell response amplitude, or temporarily disabling T-cell activation.

Conclusions and perspectives

Recent work has designed sophisticated synthetic mammalian circuits that function as programmable prosthetic networks able to act as therapeutic sensor-effector devices. These devices can sense and quantify pathological conditions, and then produce therapeutic responses to restore function. Proof-of-concept studies in animal models of human diseases demonstrate that synthetic devices such as light-wave-activated or radio-wave-activated triggers to regulate a precise therapeutic response in cells, smart logic circuits to destroy cancer cells, synthetic networks to keep metabolic homeostasis, and synthetic circuits to program immune cells all function in these contexts. These studies clearly demonstrate that synthetic biology has great potential to provide novel treatment strategies for future clinical applications [53].

However, precise interventions in multifactorial metabolic diseases will require synthetic networks with high biocomputing power, that is the ability to integrate multi-sensor activities and coordinate them with multi-output capacity [54]. The increasing complexity of synthetic gene networks needed to cope with the dynamics of metabolic diseases may exceed individual cells' engineering capacity. One way in which to achieve the

Figure 3



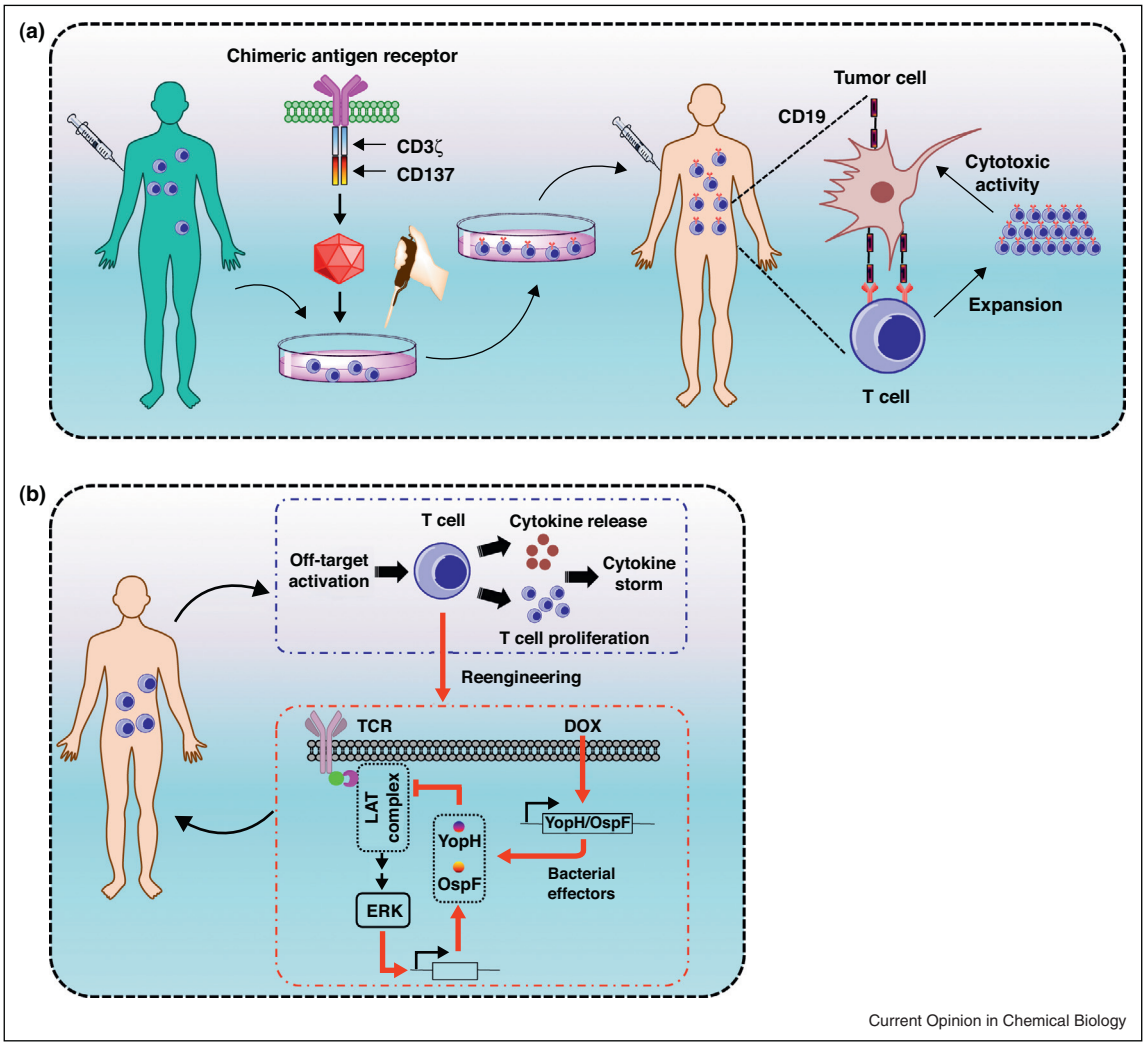
Synthetic gene circuits designed for cancer therapy. **(a)** An AND, logic-gate, cancer-killer switch. This synthetic circuit contains two sensor units, which constantly monitor the state of the two malignancy markers, and produces a kill signal. Two different tumor-specific promoters drive expression of two chimeric proteins (DocS-VP16 and Coh2-GAL4). Only when these two chimeric proteins are simultaneously expressed they assemble by dimerization to form a functional transactivator (GAL4-Coh2-DocS-VP16) that activates a specific promoter (P_{GAL4}) to trigger expression of the herpes simplex virus type 1 thymidine kinase (TK1). This transactivator converts the prodrug of ganciclovir to a toxic derivative. **(b)** A multi-input microRNA (miRNA)-based cancer classifier. This multi-input classifier is designed to only kill HeLa cells by monitoring the levels of six HeLa-specific miRNAs. This cancer classifier consists of two miRNA sensor systems. One sensor detects high levels of one set of HeLa miRNAs, and the other sensor detects low levels of another set of HeLa miRNAs. In the HeLa-high miRNA sensor, high levels of miRNA prevent expression of the reverse tetracycline-dependent transactivator (rtTA) and the repressor of the lactose operon (LacI). In the absence of LacI, the output gene (hBax) is expressed. In the HeLa-low miRNA sensor, hBax expression is inhibited by the high expression of the three HeLa-low miRNAs. This logical decision-making circuit leads to hBax expression only in HeLa cells with specific miRNA profiles.

complexity needed would be to distribute sensor and effector activities among different designer cells programmed to communicate with each other [55]. Furthermore, one could engineer human commensal bacteria to interact with designer cells and thus provide novel treatment strategies for infectious diseases and metabolic disorders [56].

Cell-based therapies are the next generation of medicine [57]. As synthetic biology progresses, scientists can engineer versatile and therapeutic cells of microbial and human origin to treat different diseases. Some multifactorial diseases, such as metabolic syndrome, may benefit from combined bacterial-mammalian cell therapy. This requires engineered inter-cellular or intra-cellular communication systems that can sense and execute precise activities.

However, before the safety questions are addressed, there is still a long way to go for clinical applications of these rationally designed, synthetic-biology, therapeutic devices. Until now, most of the synthetic gene networks

Figure 4



Synthetic signaling circuits designed for immune-mediated therapy. **(a)** Synthetic signaling cascades for chronic lymphocytic leukemia treatment. The chimeric antigen receptor (CAR) was designed by assembling four different domains: an anti-CD19 single chain fragment, a CD8α-derived transmembrane domain, a human CD137-derived co-stimulatory domain, and a CD3ζ signaling domain. The primary human T cells were isolated from the patient and transduced by lentivirus to express CAR. The CAR-transgenic T cells were then placed back into the patient. Tumor cells expressing the CD19 marker in patients could be recognized by CAR-transgenic T cells, resulting in T-cell expansion and delayed cancer progression. **(b)** Engineering kinase pathways with bacterial effectors to control the human T-cell response. Isolated primary human $CD4^+$ T cells were reengineered to control cytokine release by engineering a synthetic amplitude limiter or a synthetic pause switch. The synthetic negative feedback loop, or synthetic pause switch, was built by expressing YopH or OspF, which can inhibit specific steps in the T-cell receptor (TCR) pathway. The expression of both bacterial effectors (OspF and YopH) is under the control of a tetracycline-inducible promoter, which can be controlled by adding doxycycline. A central component of TCR signaling, the MAPK extracellular signal-regulated kinase (ERK), could be inactivated by OspF, whereas YopH can dephosphorylate the phosphotyrosine of the T-cell scaffold protein LAT. Thus, externally adding doxycycline could control T-cell activation.

are uploaded into immortalized cell lines often used in mammalian synthetic biology. It is a big challenge to make sure that the engineered cell lines containing therapeutic circuits have no side effects in humans. To minimize this risk, host cells should ideally be replaced by primary cells or stem cells that are directly isolated from the patients. The iPSC (induced pluripotent stem cell) technology has paved the way to produce and engineer autologous cells, even from elderly patients [58].

There are some arguments about the ethical and legal issues regarding clinical use of synthetic therapeutic circuits [59]. It is urgent for synthetic biologists to design and create more valuable, reliable, genetic networks to demonstrate the advantages of these innovative approaches for human therapy in clinical trials, and demonstrate their efficiency compared to traditional treatment strategies. Despite these hurdles, synthetic biology is well on its way to have a deep impact on

human health, in the forms of cell-based and gene-based therapy, regenerative medicine, and tissue and organ engineering.

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