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NEWS AND VIEWS

Synthetic gene circuits moving into the clinic

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On November 28th 2020, SyngenTech, a China-based biotechnology company, announced that the company's leading oncolytic virotherapeutic product, SynOV1.1, was approved for clinical trial by the U.S. Food and Drug Administration. After synthetic bacterial or cell therapy [1,2], this is the first introduction of a viral therapy engineered using synthetic gene circuits into the clinic. Together with the clinical development of variable living therapeutics, we anticipate that synthetic gene circuits will receive increasing attention as a revolutionary technology to improve disease-treatment efficacy.

Upon transferring into living cells, synthetic gene circuits can implement user-defined cellular functions through sensing, signaling integration and processing, and functional payload delivery. About 20 years ago, research on synthetic gene circuits began with the building of several toy circuits, such as toggle switches, oscillators, and logic gates, mostly in microorganisms [3–5]. Later, the design of gene circuits was gradually incorporated into engineered mammalian cells for therapeutic purposes [6]. Inspired by electrical circuits and natural biomolecular networks, most synthetic gene circuits conceptually consist of a sensing module, a processing module, and an output module, which can regulate the dosage, timing, and localization of gene expression and therapeutic functions in response to dynamically and heterogeneously expressed disease biomarkers (Fig.1).

The sensing module can be designed to detect intracellular, extracellular, or cell-surface signals. The

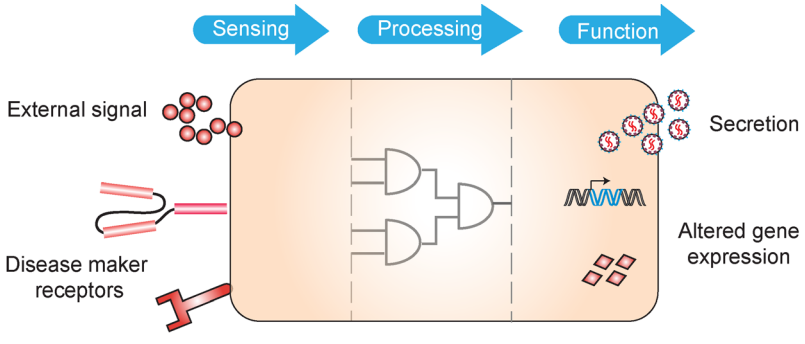

Figure 1. Synthetic gene circuits created using bio-bricks to rewire and reprogram live cells as therapeutics. In this diagram, the input is shown on the left, the gene circuit is shown in the center, and the output is shown on the right. By sensing and processing disease biomarkers, the synthetic gene circuit can control the dosage, timing, and localization of therapeutic gene expression and provide new interventions for serious diseases with unmet clinical needs.

integration of multiple signaling inputs is a fundamental strategy for improving the precision of disease targeting, and the efficacy of synthetic living drugs, thus preventing unwanted side effects. Huang *et al.* successfully engineered a synthetic oncolytic viral system that can recognize multiple intracellular cancer-specific signals (*e.g.*, promoter or microRNA) and selectively control viral replication in positive tumor cells [7]. Nissim *et al.* reported an alternative strategy for precise tumor targeting in which synthetic RNA-based circuits were deployed to release immunomodulator selectively in cancer cells in response to multiple differentially expressed transcription factors [8]. In addition, extracellular disease biomarkers were also used in the design of gene circuits. Williams *et al.* constructed a series of multi-input logic gates using synthetic Notch receptors and the chimeric antigen receptor (CAR), which can recognize multiple antigens to improve the targeting specificity of engineered T cells [9]. To regulate uric acid homeostasis *in vivo*, Kemmer *et al.* designed a synthetic gene circuit to sense uric acid levels and coordinate the expression of an engineered urate oxidase that eliminates this compound [10].

The processing module receives the signal transduced from the upstream sensing module and executes logic computation before releasing functional actions. Both open-loop and closed-loop control systems were applied to design synthetic gene circuits for the manipulation of desired therapeutic functions. Small-molecule-controlled switches were among the open-loop control methods that were used most widely to tune the output gene expression. Wu *et al.* designed a small-molecule-controlled CAR by disassembling its signal domains, which can help physicians achieve the precise titration of the activity of the CAR T cells, thereby alleviating toxicity [11]. Yin *et al.* developed a green-tea-triggered genetic control system to precisely control the time, location, and intensity of the therapeutic gene expression [12]. Compared with the open-loop control, a closed-loop system with negative-feedback control can automatically regulate therapeutic functions in response to altered output signals. For example, Ye *et al.* designed a self-adjusting synthetic gene circuit to sense and reverse the insulin-resistance syndrome based on a closed-loop control system [13].

The output module releases one or more payload molecules for programmed therapeutic functions after the correct decisions made by the control module. The synthetic oncolytic viral and RNA circuits described above can simultaneously express antibodies, cytokines, and chemokines to achieve combinatorial therapeutic efficacy, which are difficult for traditional disease treatment to achieve such curing capability.

In summary, synthetic gene circuits are constructed using modularized and standardized biological parts for sophisticated functions in live cells. Based on these synthetic gene circuits, various biotechnology start-ups, such as Synlogic, the Cell Design Labs, SyngenTech, Refuge, and Senti Bioscience, were founded and are dedicated to accelerating the development of living medicines for patients. For example, SYNB1891, which was developed by Synlogic, is an engineered bacterium that is capable of localized, targeted STING activation in phagocytic antigen-presenting cells in tumors and of promoting complementary innate immune pathways [14]. Preclinical studies have demonstrated the efficacy of SYNB1891 regarding anti-tumor immunity and long-term action. A Phase I clinical trial of SYNB1891 aimed at evaluating its safety and efficacy in patients with percutaneous accessible advanced or metastatic malignancies is ongoing (ClinicalTrials.gov Identifier: NCT04167137). As mentioned above, SynOV1.1 is an oncolytic virotherapeutic product driven by a synthetic sensory switch that can only replicate in tumor cells and secretes immunomodulators to initiate an immune response [7]. The gene circuit was designed to enhance the tumor-specific expression of the *E1A* gene and an immune effector, the human granulocyte–macrophage colony-stimulating factor (hGM-CSF), by sensing the cancer-specific alpha-fetoprotein (AFP) promoter and multiple microRNA inputs (Fig. 2). The specificity of SynOV1.1 regarding the selective killing of target tumor cells and the ability to specifically express hGM-CSF in the target cells were confirmed in nonclinical studies. SyngenTech plans to conduct a Phase I trial to characterize the safety, tolerability, and biological effects of SynOV1.1 after demonstrating its significant anti-tumor efficacy in animal models (ClinicalTrials.gov Identifier: NCT04612504).

Although there several living therapeutics exist that are being translated into the clinic stage, the engineering of gene circuits that behave as designed for preclinical studies remains a challenge. For instance, the heterogeneity and dynamical environment of the bodies of patients are largely different from those of cell cultures *in vitro*, in which the gene circuit design–build–test iterations were carried out. Moreover, the different payload genes of the output module unsurprisingly counteract each other in a competition for the limited cellular resources, thereby resulting in functional errors in the designed gene circuits [15]. Finally, cell therapies or gene therapies uploaded with the synthetic gene circuits are more complex than small molecule drugs or biomacromolecule drugs. Such complexity brings new challenges and difficulties into the manufacture, quality control, and preclinical safety evaluation of these living drugs. Therefore, there is a high demand for the establishment of a better communication between researchers in the field of synthetic biology and

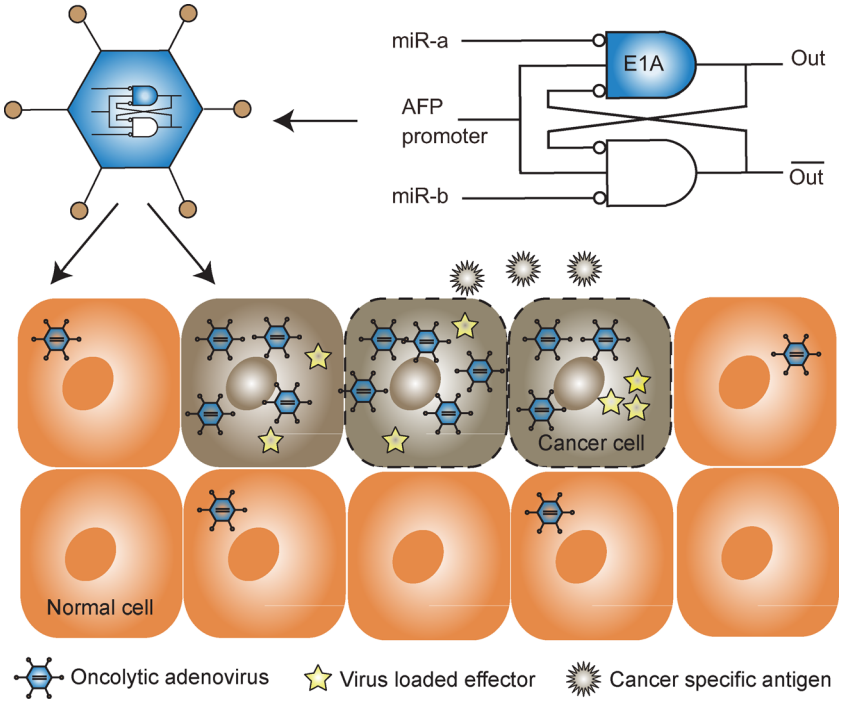

Figure 2. SynOV1.1, an oncolytic adenovirus controlled by synthetic gene circuit. Synthetic gene circuits are designed to integrate the activity of cancer-specific promoter and microRNA inputs and generate combinatorial outputs, resulting in selective viral replication in cancer cells and the modulation of immune responses. The α-fetoprotein (AFP) promoter is active in hepatocellular carcinoma cells, but not in normal liver cells. Moreover, the miR-a, a microRNA is expressed at a high level in normal cells, whereas the cancer-specific miR-b a microRNA is expressed at a high level in cancer cells. The gene circuits are triggered to activate viral replication and express immunomodulators (hGM-CSF) in cancer cells, but not in normal cells. Selective viral replication can then cause cancer cell lysis, release cancer-specific antigens, and trigger systematic anti-tumor immune responses.

government regulatory agencies regarding various aspects of the drug-development and regulatory processes. By addressing such challenges, living medicine platforms based on synthetic gene circuits will give birth to tremendous next-generation therapeutics to treat currently incurable diseases.

COMPLIANCE WITH ETHICS GUIDELINES

Zhen Xie and Huiya Huang have filed patent applications on part of work discussed in this article. Zhen Xie is a co-founder of SyngenTech. Huiya Huang owns stock in SyngenTech.

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