

COMMENTARY

Genetic control of mammalian T-cell proliferation with a synthetic RNA regulatory system - illusion or reality?

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Abstract

Synthetic RNA-based regulatory systems are used to program higher-level biological functions that could be exploited, among many applications, for *in vivo* diagnostic and therapeutic applications. Chen and colleagues have recently reported a significant technological advance by producing an RNA modular device based on a hammerhead ribozyme and successfully tested its ability to control the proliferation of mammalian T lymphocytes. Like all exciting research, this work raises a lot of significant questions. How quickly will such knowledge be translated into clinical practice? How efficient will this system be in human clinical trials involving adaptive T-cell therapy? We discuss the possible advantages of using such new technologies for specific therapeutic applications.

In the past two decades, molecular biology research has revealed the intimate mechanisms of epidemiologically significant diseases, such as cancer, infections and immunological disorders. As the next step beyond seeking the mechanisms involved, scientists are now increasingly making it possible to regulate human biological reactions. In recent years, there have been breakthroughs in genetic engineering related to the inventory and methods necessary to physically construct and assemble biomolecular parts, such as synthetic RNA-based regulatory systems [1]. Synthetic biology relies on the engineering of biological systems that perform human-defined functions and on the synthesis of complex, biologically based systems that show functions that do not exist in nature. Despite the possible

advantages for clinical applications, more work remains to be done to elucidate the principles of biological design, and to overcome the scientific and technical challenges in designing and building more effective systems that are harmless to humans and therefore useful for clinical applications.

A recent study by Chen *et al.* [2] has produced a significant advance in solving such issues and therefore potentially bridging the gap between the bench and the bedside for synthetic RNA-based regulatory systems. The authors [2] developed a modular device composed of a sensor (an aptamer) and a gene-regulatory component (a hammerhead ribozyme) and tested its ability to affect the expression of cytokines important for the function of T-lymphocytes in mouse and human systems.

Why is this work [2] significant? First, it represents the logical continuation of years of experimental work performed by the same group, coming from a team that understands the way a synthetic RNA-based regulatory system works and its immediate practical applications. In fact, in a previous study [3], also published in *Proceedings of the National Academy of Sciences of the United States of America*, the authors were the first to develop and set up universal RNA-based regulatory platforms, called ribozyme switches, by using engineering design principles. In the present report [2], the authors expanded the advantages of such biomodular platforms to a broader range of applications. They were able to do so by the reliable *de novo* construction of modular, portable and scalable control systems that can achieve flexible regulatory properties, such as up- and down-regulation of target expression levels and tuning of regulatory responses to fit application-specific performance requirements.

Second, the authors [2] applied the synthetic RNA regulatory device to a significant medical issue, the use of adoptive cell transfer (ACT) [4]. The ACT strategy uses T-cell-based cytotoxic responses to attack malignant cells (or any other types of abnormal cells) that escape the body's natural surveillance by using T cells that have a natural or genetically engineered reactivity to a patient's cancer cells. For this purpose, T cells have first to be

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naturally or genetically engineered to react against a tumor-specific antigen, then expanded and made more effective *in vitro*, and finally adoptively transferred into a cancer patient. However, the clinical efficacy of ACT, so far, has been limited. There are many reasons for this, and insufficient persistence and reactivation of infused T cells are among the main ones. Conventional strategies for enhancing the persistence of transferred T cells include ablation of all white blood cells (myeloablative methods), such as total body irradiation and administration of toxic levels of interleukin (IL)-2. However, myeloablation is associated with considerable morbidity, caused by decreased immune response and increased risk of infection [5]. Therefore, safer and more effective therapeutic strategies are yet to be discovered.

Chen *et al.* [2] report on a synthetic RNA regulatory system, which marks a new era in adoptive T-cell therapy because of the increase in the amount and survival of infused T cells found with this system. Their system for the control of mammalian T-cell proliferation is based on a platform of assembled RNA devices formed by a modular sensor (aptamer) and a gene-regulatory (hammerhead ribozyme) component. This device converts a small-molecule input to an increased gene expression output, in this particular case cytokine production. In more detail, the authors [2] fused a theophylline ribozyme switch to the 3' untranslated region of a tri-functional transgene (*cd19-tk-t2a-il15*) encoding IL-15 (potent survival/proliferative cytokine of T cells), mutant HSV-1 thymidine kinase (acting as a reporter and as a suicide protein in the presence of ganciclovir) and CD19 (a marker for fluorescence-activated cell sorting and immunomagnetic selection). Using this system, they could strictly measure (by monitoring the expression of CD19) and control (by modulating the levels of the input molecule) the biological response (cell proliferation/viability).

In addition to all the *in vitro* evidence, the authors [2] demonstrated that this system worked *in vivo* and effectively modulated the T-cell growth rate in mice in response to theophylline administration. The growth rate was increased to 32% in the presence of theophylline over a 14 day study in mice. They further investigated its possible clinical application by transducing primary human central memory T cells with this system. *In vitro* results showed that the population of live central memory T cells increased by 24% and that apoptotic cell population was decreased by 54% in the theophylline-responsive system [2].

Finally, the presented gene regulatory system [2] showed significant advantages over available gene regulatory techniques (synthetic inducible promoters); in particular, it provides a wide range of flexibility for clinical settings. Firstly, the ribozyme switches can be

easily programmed to respond to different drug molecules. Secondly, the system can be stringently controlled and finely tuned by adding additional drug-responsive ribozyme switches (up to four), therefore achieving lower basal gene expression levels. Thirdly, this system shows tight drug-mediated regulation of growth over an extended time period. Taking all these features into consideration, it is feasible that this new synthetic RNA-based regulatory system could have straightforward clinical utility.

It is likely that combining this new modular device framework with upcoming advances in synthetic biology will strongly support the tailoring of RNA-based regulatory systems to diverse applications in various clinical and laboratory environments. Yet applying these RNA-based regulatory systems in clinical practice may still require more time. In addition, there are many other factors that limit the use of adoptive T-cell therapy for cancer. For example, the failure of adoptive immunotherapy against cancers lies in the absence of tumor-specific sources of T cells [6]. If such obstacles are not overcome, efficacy of these systems will be significantly limited in clinical practice. Also, recent data support the combined roles of protein-coding genes and non-coding RNAs, such as microRNAs, in the pathogenesis of frequent diseases (such as cancer, immune and cardiac disorders) [7]. One question for the future is whether such devices can be adapted for the regulation of the functions of non-coding RNAs and microRNAs. The published research is good news, but it would be better to hold our cheers until the clinical trials are successfully completed, which we hope will be in the near future.

Abbreviations

ACT, adoptive cell transfer.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors wrote the article.

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GAC received his MD and PhD at Carol Davila University of Medicine in Bucharest, Romania. After working on cytogenetics as an undergraduate student with Dragos Stefanescu in Bucharest, he completed training in cancer genomics in Massimo Negrini's laboratory at the University of Ferrara, Italy. In 2000 he became a postdoctoral fellow at the Kimmel Cancer Center in Philadelphia, in Carlo Croce's laboratory. Since July 2007 he has been an associate professor in Experimental Therapeutics at the MD Anderson Cancer Center and studies the roles of microRNAs and other non-coding RNAs in cancer initiation and progression, as well as the mechanisms of cancer predisposition, and explores new RNA therapeutic options for cancer patients. SKL graduated from Yonsei University Medical School, Seoul, South Korea with an MD and PhD and is an assistant professor in the Department of Gastroenterology, Severance Hospital, Seoul. His primary clinical focus is treating colon and gastric cancers. The focus of his scientific research is understanding the roles of non-coding RNAs in gastrointestinal cancers. In March 2009, he began working in GAC's laboratory studying the roles of non-coding RNAs, including microRNAs in the initiation and development of gastrointestinal cancers, as well as the identification of new non-coding RNA biomarkers.

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