www.nature.com/cr
www.cell-research.com

Cell Research

RESEARCH HIGHLIGHT

When de novo-designed protein logics meet CAR-T therapies

Mingqi Xie[1,2,3,4] and Peilong Lu[1,3,5,6]

Cell Research (2020) 30:946–947; https://doi.org/10.1038/s41422-020-00419-z

Protein logic gates acting at posttranscriptional levels are unique in being amenable to the control of both intracellular and intercellular biological processes. In a recent paper published in *Science*, Lajoie et al. engineered highly versatile colocalization-dependent protein switch that are activated upon detection of logic combinations (e.g., AND, OR and NOT) of specific cell surface antigens, providing another important asset for "universal" CAR-T therapies.

In recent years, chimeric antigen receptor T cell (CAR-T) therapies based on adoptive transfer of ex vivo engineered patient-specific T cells have finally arrived on the central stage of the pharmaceutical industry.[1] Despite showing excellent response rates in many patients suffering from blood tumors (e.g., leukemia), many hurdles still need to be overcome to accelerate the development of future CAR-T therapies.[2] One major challenge is that, in many cases, multiple cell surface antigens are required to confidently distinguish tumor cells from healthy tissues.[3] In addition, selection and clinical validation of monoclonal antigen-specific CAR-T cells are highly time and resource intensive. Technologies that can avoid tumor-specific re-engineering of the T cells would therefore be heavily needed.

To address these concerns, the paper by Lajoie et al.[4] features a protein switch called Co-LOCKR (Colocalization-dependent Latching Orthogonal Cage–Key pRotein) that is specifically activated upon binding to a specific user-defined set of target antigens. Co-LOCKR is based on a de novo designed precursor switch called LOCKR,[5] which consists of a "Cage" protein that can interact intramolecularly with a terminal "Latch" helix and a specific peptide partner "Key", which can competitively bind to the Cage and release the Latch, thereby rendering a previously buried signal motif (e.g., BH3 motif from Bim) accessible to putative binding partners (e.g., Bcl-2-Bim binding). The authors optimized LOCKR by shortening the helices and designing asymmetric hydrophobic packing and hydrogen bond networks to promote specific interactions among the helices.[4] The improved LOCKR can be efficiently purified to retain full functionality in solution, and its de novo design strategy would inherently enable customizable and tunable target affinity and molecular stability.

Co-LOCKR works in a colocalization-dependent manner. The authors fused the Cage and Key to designer ankyrin repeat proteins (DARPin) to bind cancer-associated antigens (e.g., Her2, EGFR and EpCAM) with high affinity (Fig. 1). If a target cell only expresses one antigen, only one of the Cage or Key proteins can bind to the target; however, if a target cell expresses the correct combination of two antigens of interest, both Cage and Key proteins harboring the corresponding DARPins will co-localize to the target cell in proximity, resulting in activation of the protein switch. To demonstrate the utility of this combinatorial antigen detection strategy for CAR-T therapies, the authors engineered a Bcl-2-dependent CAR, which can specifically activate T-cells upon binding to Bim. Thus, Cage and Key can be used as proteinaceous adaptor molecules that are capable of screening the target tissue for the expression of "correct" combinations of cancer-associated antigens (e.g., Her2 and EGFR); only Cage is activated and allows Bim-Bcl-2 interaction when the right antigen-binding conditions are met, enabling highly programmable and precise CAR-T recruitment and activation.

While the Co-LOCKR switch design inherently encodes a molecular AND-gate, the authors went on to design additional Co-LOCKR constructs capable of performing other logic operations, such as OR and NOT. By carefully combining AND, OR and NOT operations, any type of complex Boolean calculations can be theoretically encoded. Of note, the design of protein-level logic gates is becoming increasingly important in synthetic biology owing to advantages related to programmability, direct coupling to target molecules via specific interaction or covalent linkage, fast reaction kinetics and compatibility with direct exogenous administration of purified switch components. To engineer OR-gate Co-LOCKR switches, it is possible to use the same Cage protein (e.g., specific to Her2) in combination with two different Key proteins (e.g., one specific to EGFR and the other specific to EpCAM). Thus, activation of Cage and recruitment of CAR-T can be triggered either by cells that co-express Her2 and EGFR or by cells that co-express Her2 and EpCAM. To engineer NOT-inverters, the authors introduced a third type of Co-LOCKR components named Decoy. Decoy also binds to its target antigen through a specific DARPin-motif, but acts as a sponge to sequester the Key and prevent it from activating Cage upon colocalization. Thus, Decoy is used to program typical "AND NOT" detection logics, where CAR-T recruitment is initiated upon sensing of a specific combination of target antigens (e.g., A AND B) as long as an essential "anti-target" (e.g., C) is not detected. This would dramatically help reduce issues related to on-target off-tumor toxicity of CAR-T cells in the clinics.

Co-LOCKR represents another major milestone for the so-called "universal" CAR-T strategy. To target a different tumor, conventional CAR-T strategies would necessitate the redesign of a new CAR that is specific to another antigen. In contrast, the first "universal" CAR-T cells have used an antibody-coupled T cell receptor (ACTR) system where the antigen-binding domain of a classical CAR was replaced by an antibody-binding domain CD16.[6] Thus, the same ACTR-expressing T cells could be used to target different tumors by using different antibodies in solution that are specific to different cell surface markers. To allow universal CAR-Ts

[1]Westlake Laboratory of Life Sciences and Biomedicine, Hangzhou, Zhejiang, China; [2]Key Laboratory of Growth Regulation and Translational Research of Zhejiang Province, Hangzhou, Zhejiang 310024, China; [3]School of Life Sciences, Westlake University, Hangzhou, Zhejiang 310024, China; [4]Institute of Basic Medical Sciences, Westlake Institute for Advanced Study, Hangzhou, Zhejiang 310024, China; [5]Key Laboratory of Structural Biology of Zhejiang Province, Hangzhou, Zhejiang 310024, China and [6]Institute of Biology, Westlake Institute for Advanced Study, Hangzhou, Zhejiang 310024, China
Correspondence: Mingqi Xie (xiemingqi@westlake.edu.cn) or Peilong Lu (lupeilong@westlake.edu.cn)

Published online: 25 September 2020

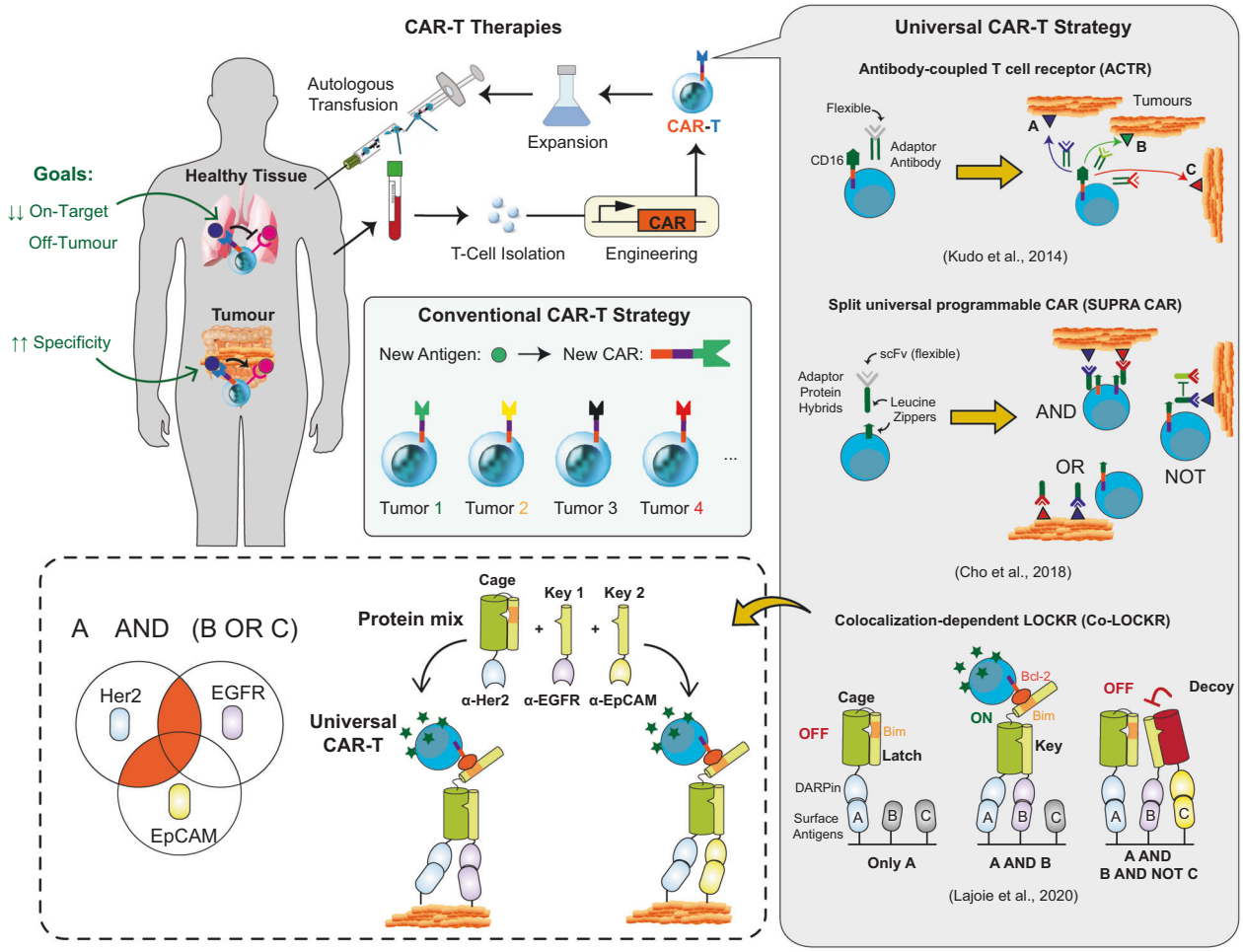

Fig. 1 Co-LOCKR, a novel solution for the universal CAR-T strategy. Major strategies of universal CAR-T therapies include ACTR, SUPRA CAR, and Co-LOCKR. To reduce on-target off-tumor toxicities, both SUPRA CAR and Co-LOCKR use protein logics to specifically target a user-defined combination of surface antigens. Mechanistically, SUPRA CAR uses naturally evolved leucine zipper interactions to create adaptor proteins, while Co-LOCKR is based on de novo protein design.

to detect different antigen combinations, a "split, universal and programmable" (SUPRA) CAR system has been developed in recent years.[2] SUPRA CAR is activated by bi-specific adaptor molecules consisting of an scFv that is specific to a target antigen (e.g., Her2) and an engineered leucine zipper domain (SYNZIP)[7] that is specific to a universal CAR-T. By carefully selecting different SYNZIP candidates with different customizable affinities, CAR-T activation could also be engineered to follow typical AND, OR and NOT logics for antigen detection. In contrast, orthogonal and tunable interaction of de novo designed Co-LOCKR proteins ensured construction of precise 3-input logics (e.g., A AND B AND NOT C), which further expanded the possible targeting precision of CAR-T cells. However, more evidence is still needed to test the safety and efficacy of the Co-LOCKR system in an animal model, as well as the immunogenicity of the artificial proteins. Nevertheless, the de novo approach[8–10] of custom-designing sophisticated CAR-T guiding adaptor proteins from scratch offers a tantalizing solution to the universal CAR-T strategy.

REFERENCES

1. Ma, C. C. et al. *Biotechnol. Adv.* **40**, 107502 (2020).
2. Cho, J. H., Collins, J. J. & Wong, W. W. *Cell* **173**, 1426–1438 (2018).
3. Roybal, K. T. et al. *Cell* **164**, 770–779 (2016).
4. Lajoie, M. J. et al. *Science* https://doi.org/10.1126/science.aba6527 (2020).
5. Langan, R. A. et al. *Nature* **572**, 205–210 (2019).
6. Kudo, K. et al. *Cancer Res.* **74**, 93–103 (2014).
7. Thompson, K. E., Bashor, C. J., Lim, W. A. & Keating, A. E. *ACS Synth. Biol.* **1**, 118–129 (2012).
8. Chen, Z. et al. *Nature* **565**, 106–111 (2019).
9. Chen, Z. et al. *Science* **368**, 78–84 (2020).
10. Huang, P.-S., Boyken, S. E. & Baker, D. *Nature* **537**, 320–327 (2016).