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An invited review following *the Soujinkai Award*: Development of Novel Chimeric Antigen Receptor-expressing T Cells Promoting Immune Cell Infiltration and Survival in the Tumor Tissue

Keishi Adachi and Koji Tamada

Department of Immunology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
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Correspondence to Keishi Adachi, D.V.M., PhD. E-mail: fairkid@yamaguchi-u.ac.jp

Abstract Chimeric Antigen Receptor (CAR) -T cell therapy is attracting the attention for its remarkable therapeutic efficacy on hematological malignancies. Meanwhile, some serious issues currently remain in CAR-T cell therapy, one of which is the ineffectiveness against solid tumors. In order to overcome the issue, we developed the next generation CAR-T cell which simultaneously produce IL-7 and CCL19 (7 × 19 CAR-T cell). In several solid tumor mouse models, administration of 7 × 19 CAR-T cells induced conspicuous infiltrations of T cells and dendritic cells (DCs) inside the tumor tissues, and exert potent antitumor efficacies in cooperation with the recipient T cells. In addition, a long-term immunological memory against the tumor was formed after the treatment, suggesting that the treatment with 7 × 19 CAR-T cells enabled the prevention of cancer recurrence. In conclusion, our study concerning the next-generation CAR-T cell is expected to lead an innovative therapy against solid tumors.

Key words: solid tumor, T cell, CAR-T cell, IL-17, CCL19

Immunotherapy against cancer

Since 1981, malignant neoplasms have been the leading cause of death in Japan, and the increasing trend has been continuing. The Japanese Ministry of Health, Labor and Welfare (MHLW) reported that the ratio of malignant neoplasms to the total number of deaths in 2018 was 27.4%. Therefore, it should be imperative to develop effective methods for therapies against cancers and for preventions of the recurrence.

Cancer immunotherapies have been investigated as treatments for refractory or progressive malignancies for which three conventional standard therapies, i.e., surgery, chemotherapy, and radiotherapy, are hard to be applied. In recent years, clinical development of immune checkpoint inhibitor (ICI) has rapidly progressed. In March 2011, ipilimumab (trade name: Yervoy™), anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein-4, CD152) monoclonal antibody, was approved for the first time in the world by the US Food and Drug Administration (FDA) to treat patients with late-stage melanoma that has spread or cannot be removed by surgery.[1] Nivolumab (trade name: Opdivo®), anti-PD-1 (programmed cell death-1, CD279) monoclonal antibody, was first approved in July 2014 by MHLW as a treatment for unresectable melanoma.[2] Currently, the types of cancers to which nivolumab is applicable continues to increase in many countries including Japan. Furthermore, as a new immunotherapy following ICI, CAR-T cell therapy, in which

T cells are endowed with strong tumoricidal efficacies by the transfection of CAR specific to a cancer antigen, has been developed, and its outstanding therapeutic efficacies have attracted much attention.[3,4]

Molecular structure of CAR

CAR is an engineered antigen receptor consisted of three components; an immunoglobulin single-chain variable fragment whose light chain (VL) and heavy chain (VH) are derived from a monoclonal antibody specific to a cancer cell surface antigen, a transmembrane domain, and intracellular signaling domains (Fig. 1).[5] In many cases, CAR-T cells are generated by transfecting CAR gene into peripheral blood T cells with a lentiviral or retroviral vector. CAR-T cells have a potential to detect and attack tumor cells without the restriction of HLA, which is a major difference from normal T cells. Since the reactivities of CAR-T cells to cancer are bestowed by gene transfer, large numbers of CAR-T cells can be produced independently of endogenous T cells reactive to the cancer, which are considered to be induced only a small amount in the patient. In addition, since CAR-T cells are generated with T cells that are not immunologically exhausted, the potent antitumor efficacies can be maintained. CAR constructs are categorized into three by the structure of intracellular signaling domains; "first generation" consists of only a CD3ζ (CD247), "second generation" does of one costimulatory molecule, such as CD28 or CD137 (4-1BB), in addition to CD3ζ, and "third generation" does of multiple costimulatory molecules in addition to CD3ζ. It has been reported that second and third generation CAR-T cells are superior to first generation ones in proliferation, cytokine production, cytotoxic activity, and in vivo viability.[5]

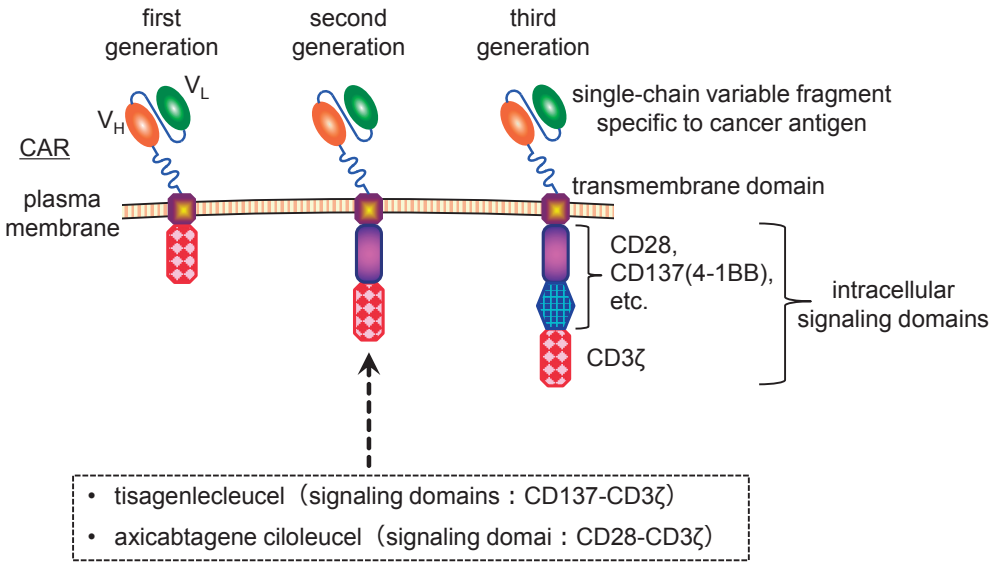

Fig. 1 Molecular structure of CAR

CAR is an engineered antigen receptor consisted of three components; an immunoglobulin single-chain variable fragment whose light chain (V_L) and heavy chain (V_H) are derived from a monoclonal antibody specific to a cancer cell surface antigen, a transmembrane domain, and intracellular signaling domains. First generation CAR" consists of only a CD3ζ (CD247), second generation CAR does of one costimulatory molecule, such as CD28 or CD137, in addition to CD3ζ, and third generation CAR does of multiple costimulatory molecules in addition to CD3ζ. Second or third generation CAR-T cells were employed in most of the current clinical trials, and both tisagenlecleucel and axicabtagene ciloleucel belong to second generation.

Clinical development of CAR-T cell therapy

Professor Carl H. June (the University of Pennsylvania) and his colleagues developed tisagenlecleucel (trade name: Kymriah™), an anti-CD19 CAR-T cells, in collaboration with Novartis Pharmaceuticals, and they conducted clinical trials for multiple B cell malignancies.[3,4] In 30 child or adult patients with relapsed or refractory acute lymphoblastic leukemia (ALL), the complete remission rate was 90%, the recurrence-free survival rate at 6 months was 67%, and the overall survival rate was 78%, demonstrating the remarkable therapeutic efficacies of CAR-T cell therapy. On the other hand, adverse events assumed to be caused by the injection of CAR-T cells were also reported. Cytokine release syndrome occurred in almost all patients early after the administration. In 8 cases, severe symptoms, such as hypotension requiring the treatment with vasopressor agent or respiratory failure requiring ventilator, were observed. However, those symptoms were improved by the administration of tocilizumab, an anti-IL-6 receptor monoclonal antibody (trade name: Actemra®).[6] Based on those results, in August 2017, FDA first approved tisagenlecleucel in the world for the patients with refractory or relapsed B cell ALL under 25 years of age. Following the first approval, FDA approved tisagenlecleucel also for adult relapsed or refractory diffuse large B-cell lymphoma (DLBCL) in May 2018. Tisagenlecleucel was approved in EU and Japan in 2018 and 2019, respectively, for the two types of B cell malignancies described above. In addition, FDA approved axicabtagene ciloleucel (trade name: Yescarta®) developed by Kite Pharma (acquired by Gilead Sciences in August 2017), which is another anti-CD19 CAR-T cell, for the adult patients with relapsed or refractory DLBCL in October 2017.[7,8] As of December 2019, many clinical trials with CAR-T cells, mainly with anti-CD19 CAR-T cells, are ongoing in the world.

Problems of current CAR-T cells and the next generation CAR-T cells

Unfortunately, the current CAR-T cell therapy has not yet been definitive, and some crucial issues remain. One of the problems to be overcome is the ineffectiveness against solid tumors, which accounts for the majority of malignancies. It has been pointed out that one reason for the ineffectiveness is the lack of the technology to accumulate and proliferate intravenously injected CAR-T cells in tumor tissues.[9,10] We research and develop CAR-T cells that exert potent therapeutic effects against solid tumors by endowing the cells with the capacities to control immune functions inherent in patients, and those next generation CAR-T cells are referred to as "Prime CAR-T cells (Proliferation-inducing and migration-enhancing CAR-T cell)." We have recently developed and reported one kind of Prime CAR-T cells[11] bestowed the ability to simultaneously produce IL-7, which stimulates survival and proliferation of T cells,[12,13] and CCL19, which induces migration of T cells and DCs.[14,15] Previous studies have demonstrated that IL-7 and CCL19 were important for the formation of the structure of T-cell zone of lymph nodes, where T cells and DCs accumulated.[16,17] Based on those findings, we hypothesized that conferring the ability to concomitantly produce IL-7 and CCL19 upon CAR-T cells would induce the accumulation of T cells and DCs inside tumor tissues like lymph nodes, resulting in the exertion of potent anticancer efficacies. We refer to this type of Prime CAR-T cells as "7×19 CAR-T cells" (Fig. 2a).[11]

In order to investigate the antitumor effects of 7×19 CAR-T cells, we developed a mouse model, in which a mastocytoma line P815 transfected with human CD20 (hCD20, P815-hCD20) as a surrogate antigen was subcutaneously inoculated on syngeneic DBA/2 mice. After the formation of tumor mass was grossly confirmed, anti-hCD20 conventional or 7×19 CAR-T cells were intravenously administered into the tumor-bearing mice. Treatment with the conventional CAR-T cells little controlled the tumor outgrowth, resulting that most mice were eventually killed (Fig. 2b). In contrast, complete regression of the tumor was induced in most of the mice after the administration of 7×19 CAR-T cells, and those mice survived for more than 140 days (Fig. 2b). Similar results were obtained in lung and pancreatic cancer models. These

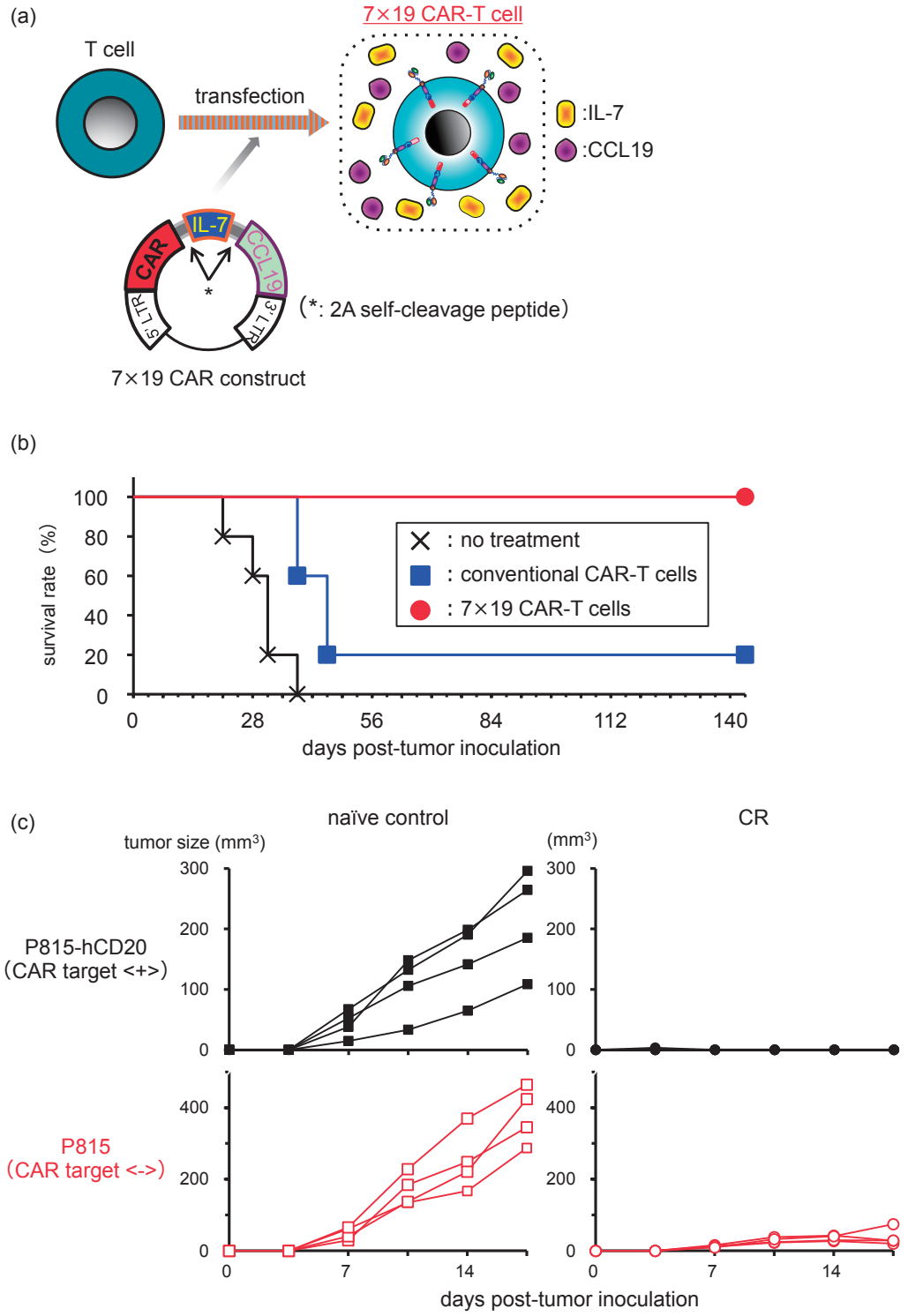

Fig. 2 Potent antitumor efficacies of 7×19 CAR-T cells against solid tumor

7×19 CAR construct that simultaneously expresses CAR, IL-7, and CCL19 in one cell by taking advantage of 2A peptide was created, and was transduced into primary T cells (7×19 CAR-T cells). (b) P815-hCD20 was subcutaneously inoculated on syngeneic DBA/2 mice. After the formation of tumor mass was grossly confirmed, anti-hCD20 conventional or 7×19 CAR-T cells were intravenously administered into the tumor-bearing mice. Survival of the mice were assessed periodically. (c) Naïve control and the tumor-rejected mice (CR) were re-challenged with P815-hCD20 and the parental P815 on the left and right flanks, respectively. The volumes of the tumors were assessed.

results suggested that 7×19 CAR-T cells were much superior to conventional ones in therapeutic capacities against solid tumors.

We next conducted histopathological examination of the tumors resected after the administration of 7×19 CAR-T cells, and found significant infiltration of T cells and DCs.[11] In order to further characterize tumor-infiltrating T cells induced by 7×19 CAR-T cell therapy, we generated 7×19 CAR-T cells with congenic mice, and treated the tumor-bearing mice with the 7×19 CAR-T cells. Immunohistochemistry with the tumor tissues resected from those mice displayed that not only donor-derived but also host-derived T cells were remarkably infiltrated into the tumor tissues.[11] Based on this finding, we depleted host T cells with an antibody specific to host T cells, and found an attenuation of the antitumor effects of 7×19 CAR-T cells.[11] These results suggested that 7×19 CAR-T cells induced infiltration and proliferation of host T cells and DCs as well as of the administered CAR-T cells, and that the CAR-T cells and host immune cells collaborated to exert synergistically antitumor activities.

Next, naïve control mice and the mice which had rejected P815-hCD20 by the treatment with anti-hCD20 7×19 CAR-T cells were re-challenged not only with P815-hCD20 on the left flank but also with hCD20-negative parental P815 on the right flank. In contrast to uncontrollable outgrowth of those tumors on naïve mice, the tumor-rejected mice completely dampened the growth of P815-hCD20, suggesting the efficient memory formation against the tumor after the treatment with 7×19 CAR-T cells (Fig. 2c). Interestingly, the tumor-rejected mice were resistant also to parental P815 (Fig. 2c). This indicated that epitope-spreading was induced in the process of eliminating P815-hCD20 after the administration of anti-hCD20 7×19 CAR-T cells. It was assumed that immunogenic cell death of tumor cells induced by 7×19 CAR-T cells via the interaction between the CAR and its target triggered the activation of host T cells specific to endogenous cancer antigens, and that both the CAR-T cells and tumor-reactive host T cells formed a memory against the tumor after the rejection.[11] It is well-known that the expression of molecules that can be CAR targets is spatiotemporally instable in solid tumors, and this is one of the reasons why CAR-T cell therapy is not effective against solid cancers.[18] Therefore, the induction of epitope-spreading is considered to be important in overcoming the issue above.

Conclusion

Conventionally, the function of CAR-T cells has been focused only on the direct tumoricidal ability. However, our studies suggest that Prime CAR-T cells, such as 7×19 CAR-T cells, can play roles not only as direct antitumor effector cells but also as delivery systems to convey immune-regulatory molecules, by which immune responses against solid tumors can be triggered, augmented and sustained in vivo. This is a paradigm shift that greatly changes the concept of CAR-T cells (Fig. 3). It is expected that Prime CAR-T cell technology leads to the expansion of clinical usability of CAR-T cells.

Conflict of interest

Keishi Adachi declare no conflict of interest.

Koji Tamada hold stocks of Noile-Immune Biotech Inc.. Koji Tamada receive consulting fees from Noile-Immune Biotech Inc..

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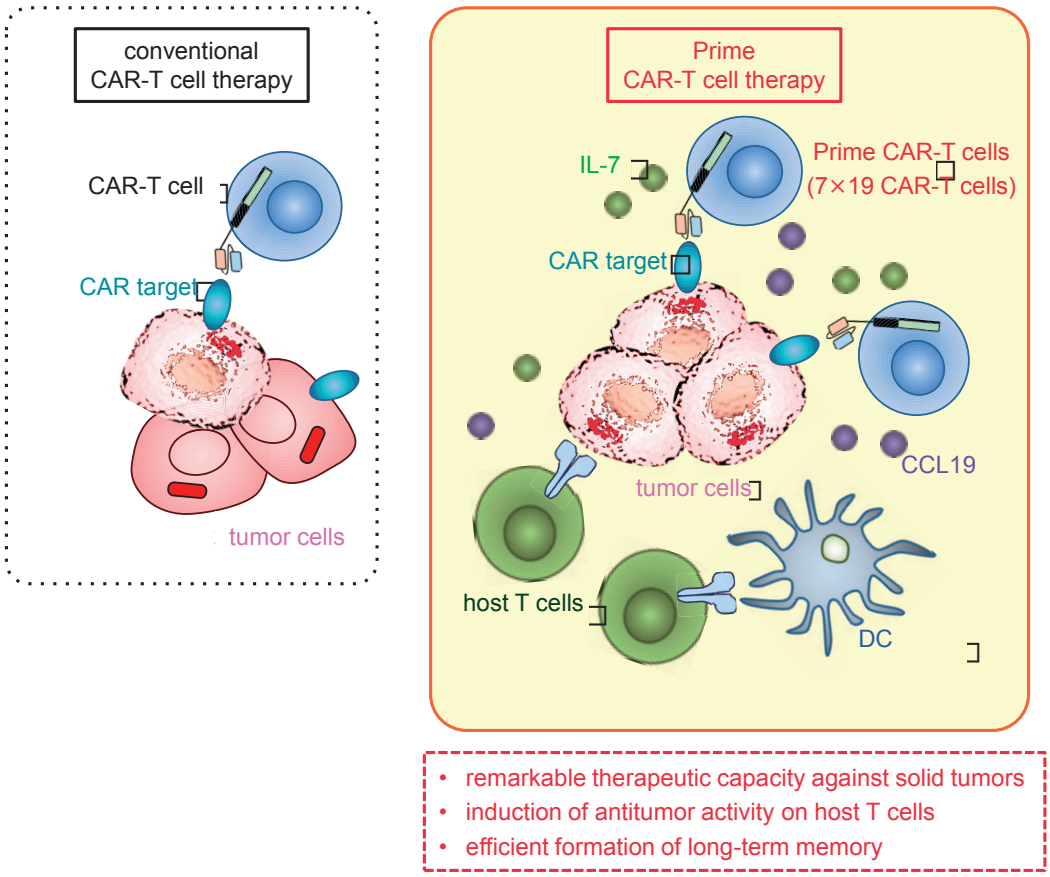

Fig. 3 The mechanisms by which Prime CAR-T cells (7×19 CAR-T cells) exert therapeutic effects against solid tumors

Administration of 7×19 CAR-T cells leads the massive infiltration not only of donor CAR-T cells but also of host T cells and DCs into the tumor tissues. Consequently, epitope-spreading is induced, and the donor cells and host immune cells collaborate to maximize the antitumor activities. Moreover, it is assumed that the recurrence of the tumor is inhibited by efficient formation of immunological memory.

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