News Release

Newly developed CAR-T cells enhance proliferation and survival compared to conventional CAR-T cells

Key Points

・ Proliferation and persistence of CAR-T cells in vivo are critical for therapeutic success after chimeric antigen receptor (CAR)-T therapy for patients with hematological malignancy.

・We performed a genome-wide CRISPR screening in CD19CAR-T cells and discovered that CUL5 knockout (KO) enhanced CAR-T cell proliferation.

・In B cell lymphoma bearing mouse model, CUL5KOCAR-T therapy showed better anti-tumor effects than conventional CAR-T therapy

・Using a novel lentiviral vector in which CUL5 shRNA is linked to the construct of CD19CAR, CUL5 knockdown CAR-T cells may improve survival rate compared with conventional CAR-T cells in the same manufacturing process as before.

Summary

Graduate student Yoshitaka Adachi (1st author), Lecturer Seitaro Terakura (Correspondence), and Professor Hitoshi Kiyoi of the Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Associate Professor Yoshitaka Sato of the Department of Virology, Nagoya University Graduate School of Medicine, and Professor Yusuke Okuno of the Department of Virology, Nagoya City University Graduate School of Medicine, found that the CUL5 gene regulates the proliferation of chimeric antigen receptor (CAR) T cells. Deficiency of CUL5 gene resulted in better proliferation of CAR-T cells, which showed superior anti-tumor effects compared to conventional CAR-T cells in the mouse model with malignant lymphoma.

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Recently, CD19CAR-T cells genetically modified to express CD19CAR targeting CD19 have shown promising results in the patients with relapsed/refractory B-cell malignancies. However, despite high initial response rates, many patients eventually relapse, suggesting that poor proliferation and long-term persistence of CAR-T cells in vivo are associated with poor responses and early relapse. To identify genes involved in CAR-T cell proliferation, we performed genome-wide CRISPR screening. We found that the CUL5 gene regulates CAR-T cell proliferation, and that functional

deficiency of CUL5 enhances the JAK-STAT signaling pathway, a signal important for T cell proliferation. CUL5 knockout (KO) CAR-T cells suppressed tumor growth in the B cell lymphoma model compared to conventional CAR-T cells. Furthermore, we developed CUL5-knockdown (KD) CD19CAR-T cell that can be generated in the same process as conventional CAR-T cell production by using a novel lentiviral vector containing shRNA against CUL5 linked to the CD19CAR construct. This therapy also enhanced anti-tumor effects. CUL5KD-CD19CAR-T cells have the potential to sustain complete remission in the patients with B cell lymphomas and ultimately improve therapeutic outcomes. This work was published in the international scientific journal "Nature Communications", on December 10, 2024.

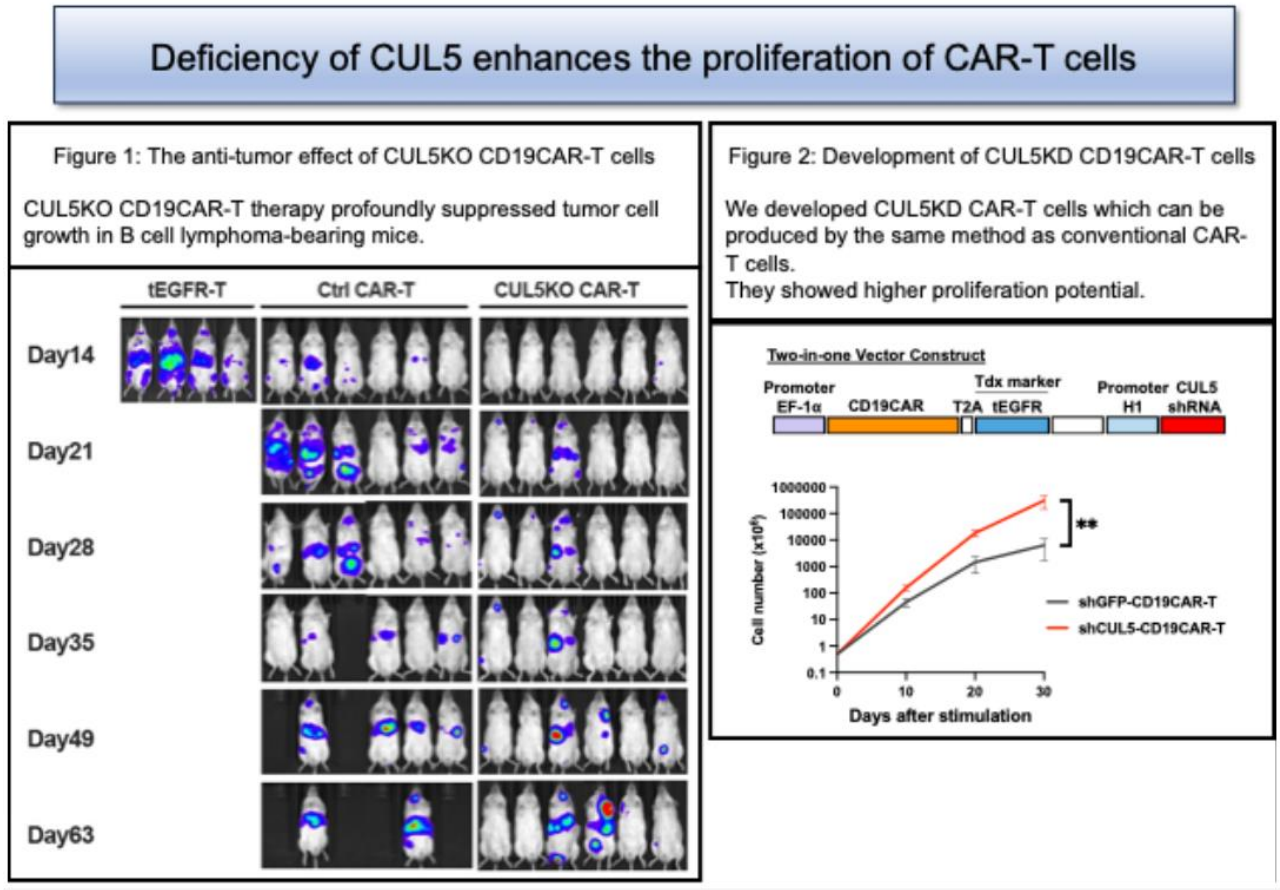
Research Background

CD19CAR-T cells, genetically engineered to express CD19-targeted CAR, have shown promising results in relapsed/refractory B-cell malignancies. However, half of the patients with acute lymphocytic leukemia treated with CD19CAR-T therapy relapse after remission, and the response rate is even lower in patients with B cell lymphoma. The poor CAR-T cell proliferation and long-term persistence in vivo are correlated with incomplete response and early relapse. We attempted to identify genes involved in CAR-T cell proliferation using a genome-wide (GW) CRISPR knockout (KO) screening method.

Research Results

We applied GW-CRISPR-KO screening system to human CAR-T cells. Pooled KO-CD19CAR-T cells were generated, and they were cultured in vitro with repeated antigen stimulation with CD19-positive tumor cells, and the number of sgRNA reads was compared before and after repeated stimulation. The CUL5 gene knockout resulted in significantly better proliferation of CD19CAR-T cells in response to repeated antigen stimulation. CUL5 KO increased the effector-memory cell fraction of CAR-T cells and significantly better cytokine production after antigen stimulation. Gene expression analysis showed that CUL5KO-CAR-T cells showed upregulated gene expression of the JAK-STAT signaling pathway compared to conventional CAR-T cells. Furthermore, CUL5KO-CD19CAR-T therapy profoundly suppressed tumor cell growth in B cell lymphoma-bearing mice, leading to long-term effective tumor clearance and prolonged overall survival compared to conventional CAR-T cells (Figure 1). CUL5KO-CD19CAR-T cells showed increased expression of phosphorylated STAT3 after co-culture with tumors, while CUL5 knockdown KHYG-1 cells showed increased expression of phosphorylated JAK1 and phosphorylated JAK3.

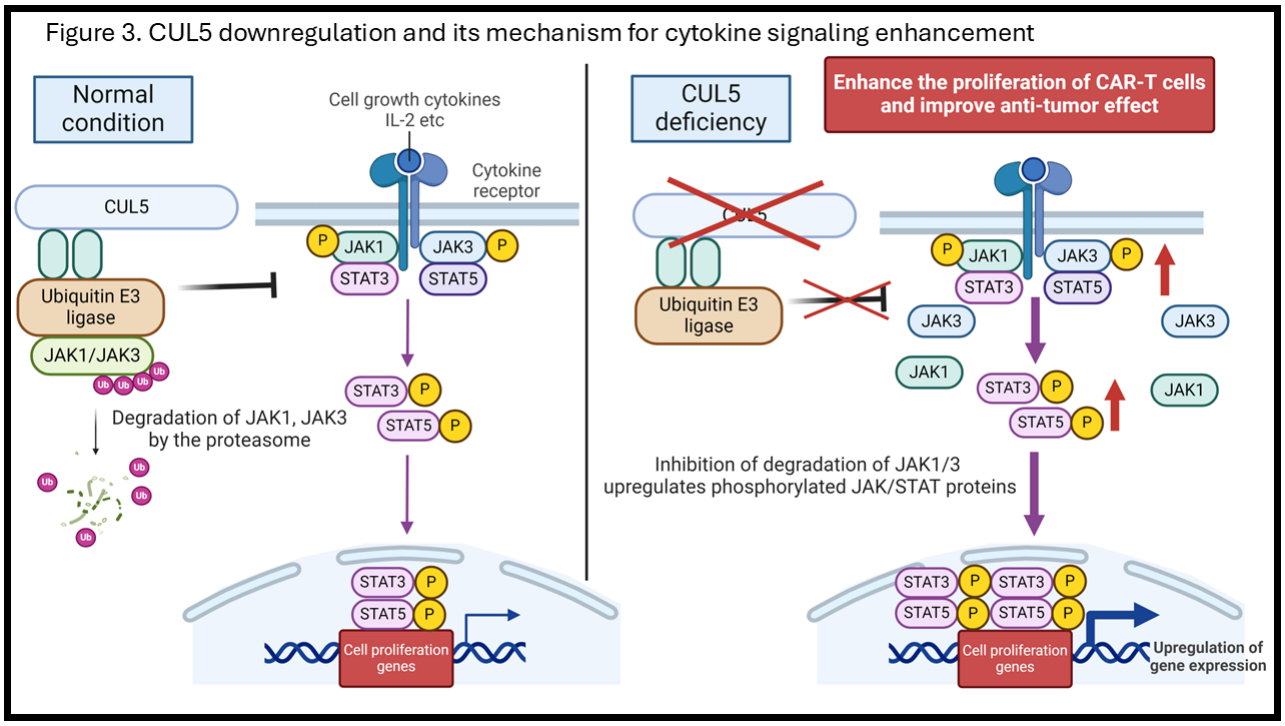
Since CUL5 is a ubiquitin E3 ligase, we can expect that the degradation of JAK protein was prevented in CUL5KO-CAR-T cells, resulting in the enhanced expression of pSTAT3.



The generation of CUL5KO-CD19CAR-T cells requires electroporation, which is difficult to apply clinically due to its toxicity and inability to harvest sufficient number of cells. Therefore, we tried to develop the lentiviral plasmid containing a CUL5 shRNA linked to CD19CAR and generate CUL5 knockdown (KD)-CD19CAR-T cells in the same method as conventional CAR-T cell generation. The CUL5KD-CD19CAR-T cells showed extremely high proliferative capacity in vitro (Figure 2) and significantly suppressed subcutaneous tumor growth in tumor bearing mouse model. Based on the findings above, a patent application was filed (International Publication No.: WO2023/228968, International Publication Date: November 30, 2023).

Research Summary and Future Perspective

We clarified the function of CUL5 in CAR-T cells, and demonstrated that deficiency of CUL5 enhanced the proliferation of CD19 CAR-T cells (Figure 3). The manufacturing process of CUL5KD-CD19CAR-T cells is as simple as the one of conventional CD19CAR-T cell and might have the potential to sustain remission and prolong survival for patients with B cell lymphoma. In addition, we have been making efforts to apply CUL5KO CAR-T cells to other target antigens because modulation of CUL5 might be promising approach in CAR-T therapy for solid tumors, which currently have limited therapeutic efficacy.



Publication

Yoshitaka Adachi, Seitaro Terakura, Masahide Osaki, Yusuke Okuno, Yoshitaka Sato, Ken Sagou, Yuki Takeuchi, Hirofumi Yokota, Kanae Imai, Peter Steinberger, Judith Leitner, Ryo Hanajiri, Makoto Murata, and Hitoshi Kiyoi, Cullin-5 deficiency promotes chimeric antigen receptor T cell effector functions potentially via the modulation of JAK/STAT signaling pathway, Nature Communications (2024),
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Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Nat_241210.pdf