



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Autophagy Inhibition Prevents CAR-T Exhaustion and Terminal Differentiation Via TCF7 AccumulationXinyi Teng¹, Mi Shao¹, Xin Guo², Tao Cheng^{3,4}, Pengxu Qian, PhD^{5,6,7}, He Huang^{8,9,10}¹ Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Med, Hangzhou, CHN² Bone Marrow Transplantation Center, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China³ Tianjin Institutes of Health Science, Tianjin, China⁴ State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences&Peking Union Medical College, Tianjin, China⁵ Institute of Hematology, Zhejiang University, Hangzhou, China⁶ Liangzhu Laboratory, Zhejiang University, Hangzhou, China⁷ Zhejiang Province Engineering Laboratory for Stem Cell and Immunity Therapy, Zhejiang University, Hangzhou, China⁸ Zhejiang Province Engineering Laboratory for Stem Cell and Immunity Therapy, Hangzhou, China⁹ Liangzhu Laboratory, Zhejiang University Medical Center, Hangzhou, China¹⁰ Bone Marrow Transplantation Center, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Introduction: Chimeric antigen receptor T (CAR-T) cell therapy has achieved remarkable success in hematological malignancies. However, CAR-T cell dysfunction due to exhaustion has been identified as a major roadblock to effective CAR-T cell therapy. Autophagy mediates T cell differentiation, survival, and memory by regulating the degradation of organelles and specific proteins. A recent study reported that inhibition of autophagy significantly enhanced the killing effect of CD19 CAR-T cells. Yet, the impact of autophagy inhibitors on CAR-T cell exhaustion and maintenance is still unclear. The study aims to systematically evaluate the role and mechanism of autophagy inhibitors on CAR-T cells.

Methods and Results: To explore whether autophagy inhibitors could prevent CAR-T cell exhaustion triggered by antigen-independent CAR tonic signaling, we applied 3 autophagy inhibitors: SBI0206965, MRT67307, Compound C, respectively, to the culture medium of CD19.4-1BBz CAR-T cells. We found that all the 3 autophagy inhibitors could reduce CAR-T cell exhaustion and terminal differentiation. The results were replicable with GD2.28z CAR-T cells. Among the three inhibitors, SBI0206965 showed the most significant effect. Thus, we chose SBI0206965 for further research. CD19.4-1BBz CAR-T cells pretreated with SBI0206965 demonstrated enhanced killing capacity against Nalm-6 cells in vitro. Using the Nalm-6-bearing leukemia xenograft model, we found that compared to DMSO pretreated CD19.4-1BBz CAR-T cells, SBI0206965 pretreated CD19.4-1BBz CAR-T cells showed superior persistence and antitumor efficacy, exhibited a less exhausted and differentiated state, and further prolonged mice survival (Fig.1). To evaluate whether SBI0206965 could protect CAR-T cell exhaustion triggered by antigen stimulation, we cocultured CD19.4-1BBz CAR-T cells with Nalm-6 cells in the medium with or without SBI0206965. We demonstrated that the addition of SBI0206965 could effectively inhibit CAR-T cell exhaustion and terminal differentiation driven by antigen exposure. By performing proteomics, we identified TCF7 enriched in SBI0206965-treated cells. We validated the differences using Western blot analysis, which showed increased TCF7 levels in CAR-T cells treated with SBI0206965 for 24 hours. Thus we speculated that inhibition of autophagy might contribute to the accumulation of TCF7 and further improve CAR-T maintenance. To further verify whether TCF7 was degraded in an autophagy-dependent manner, we next determined TCF7 turnover rates in the presence of cycloheximide (CHX) in CAR-T cells with or without SBI0206965 treatment. CHX chase assay showed that SBI0206965 treatment could slower the TCF7 degradation rate(Fig.2).

Significance: Our research provides a strategy to optimize CAR-T antitumor efficacy and persistence via TCF7 accumulation by autophagy inhibition.

Disclosures No relevant conflicts of interest to declare.

Figure 1

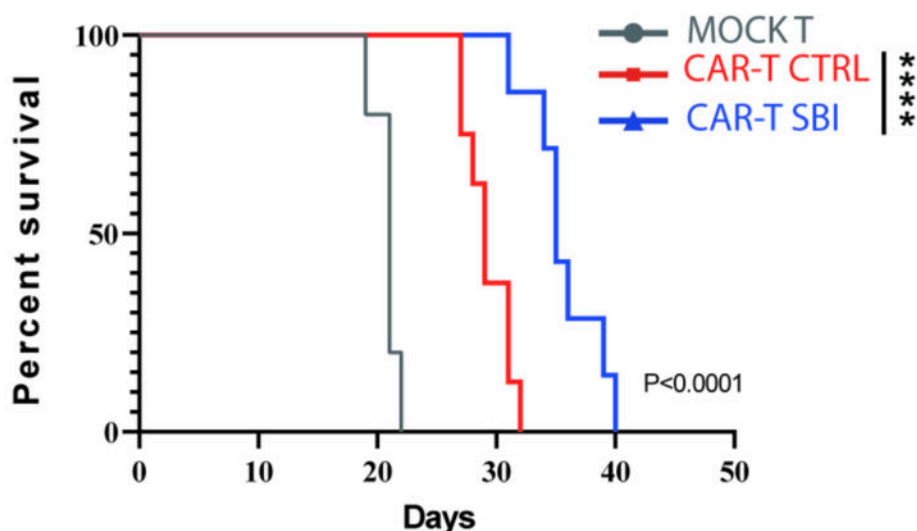


Figure 2

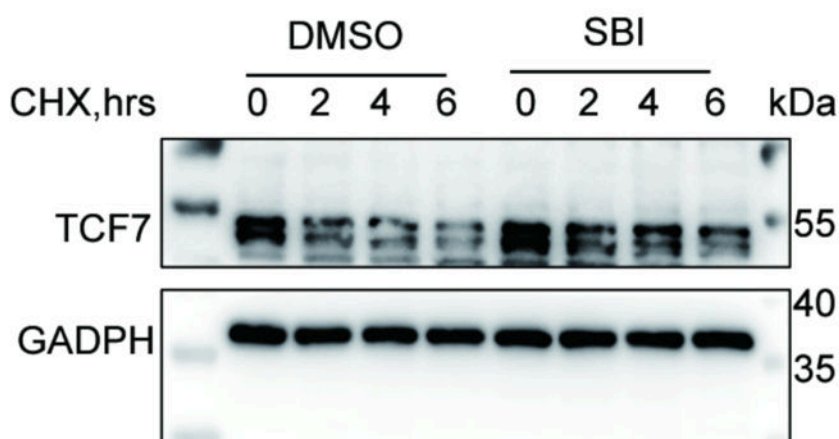


Figure 1. Kaplan-Meier analysis of survival of mice. CAR-T cells were cultured with SBI0206965 or DMSO for 3 days and then adoptively transferred to mice bearing Nalm-6 leukemia cells.

Figure 2. CAR-T cells were cultured with SBI0206965 or DMSO in the presence of cycloheximide (CHX) (500 ng/ml) for indicated time points, detected by immunoblotting with TCF7 and GAPDH antibodies.

Figure 1

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