mRNA encoding a CAR specific stimulating protein (CSSP) mediates tumor metabolism regulated (TMR) CAR signaling for CAR-T expansion beyond the tumor microenvironment

Frederic Vigant, Ani Kundu, Michael Betts, Gregory I. Frost, & Sid P. Kerkar

Abstract

Background: TMR Chimeric Antigen Receptors, with binding restricted to antigen in the tumor microenvironment, lack CAR-dependent stimulatory signals for proliferation outside the tumor. Increased exposure to CAR-T is associated with improved clinical outcomes and therefore tools to safely and selectively increase proliferation represent potential adjunctive CAR-T therapeutics. Therefore, we developed a surrogate CAR Specific Stimulating Protein (CSSP) approach encoded by synthetic mRNA for expression in antigen-presenting cells to boost CAR-T proliferation in vitro and in vivo.

Methods: NFAT-Luc reporter lymphocytes were transduced with lentivectors encoding TMR CARs with or without epitopes reactive to CSSP's in the CAR. Subsequently, PBMCs were transfected with LNPs containing synthetic mRNAs for either GFP or candidate membrane-bound CSSPs. Transfection efficiency was confirmed by microscopy and flow cytometry. The activity of CSSP's expressed in monocytes cultured with T cells expressing CAR variants with NFAT-luc reporter was measured by luciferase expression and compared to CD3 stimulation. Additionally, the kinetics of CAR stimulation with monocytes expressing CSSPs were determined by serial luciferase measurements. Finally, the proliferation of PBMCs transduced with a TMR-HER2 CAR co-cultured with CSSP-treated purified monocytes was evaluated.

Results: Following exposure of PBMCs expressing CSSPs to TMR-CAR-expressing NFAT T cells, CAR-specific luciferase signaling was readily detectable compared to non-specific CD3 stimulation, indicating that the CSSPs were capable of CAR-specific stimulation through the CD3 zeta chain of the CAR. Two of the CSSP variants demonstrated stimulation in a CAR-specific fashion at levels similar to or greater than non-specific CD3 stimulation. In kinetic experiments, stimulation of NFAT could be detected over a period of 8 days, indicating persistence of the CSSP similar to GFP in antigen-presenting cell controls. Finally, co-culture of CSSP mRNA expressed in purified monocytes were capable of TMR-HER2-CAR T cells stimulation, which do not proliferate when cultured with HER2 antigen-expressing cells under physiologic conditions.

Conclusion: TMR-CAR-T cells can achieve proliferative capacity outside the tumor microenvironment using synthetic mRNA encoding CAR-specific Stimulatory Proteins. This novel mRNA approach may provide additional tools to promote the proliferation and/or persistence of solid tumor-targeted CAR-T cells outside the tumor environment.

Fig. 1. Screening of CSSP variants for CAR NFAT activation

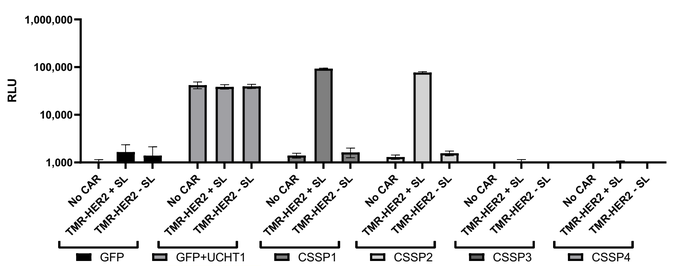

Figure 1: Recognition of a specific synthetic CAR Ligand by the CAR specific stimulating proteins (CSSP). PBMCs were transfected with the different CSSPs, and GFP as a control. 24h later the transfected PBMCs were exposed to Jurkat-NFAT reporter cells (Promega) previously transduced with lentiviral vectors to express the TMR-HER2 CARs, with or without the specific ligand (SL) for the CSSPs. UCHT1 (500 ng/mL) was used as a control of the activation of the Jurkat-NFAT-CAR cells. Only CSSP1 and CSSP2 effectively recognized and stimulated the TMR-HER2+SL CAR. n=2 +/-SD.

Fig. 2. CSSP1 can activate CAR-T cells

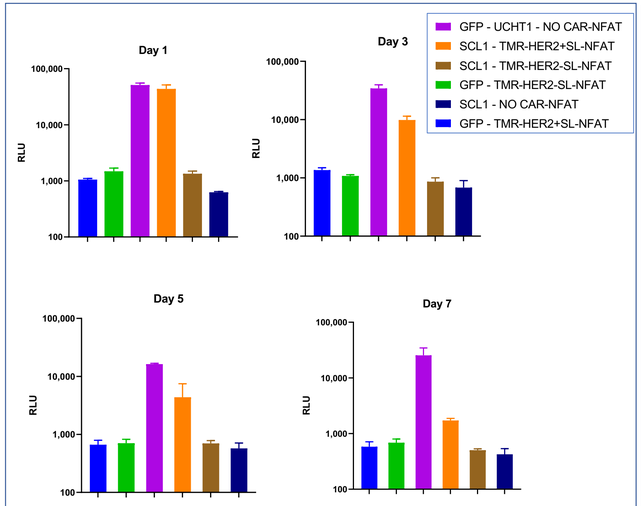

Figure 2: Kinetics of the NFAT activation of CAR-expressing T cells. PBMCs were transfected with ~~the~~ CSSP1, and GFP as a control. 24h later the transfected PBMCs were exposed to Jurkat-NFAT reporter cells (Promega) previously transduced with lentiviral vectors to express the TMR-HER2 CARs, with or without the specific ligand (+/-SL) for the CSSPs. UCHT1 (500 ng/mL) was used as a control of the activation of the Jurkat-NFAT-CAR cells. The data suggests that the stimulation of the TMR-HER2+SL CAR can occur over several days. n=2 +/-SD.

Fig. 3. CSSP1 increases CAR-T expansion

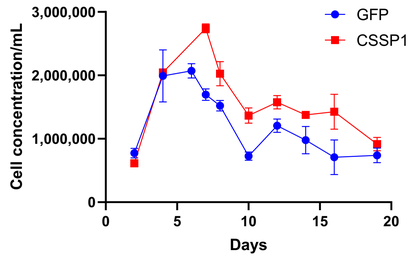

Figure 3: Stimulation (activation and expansion) of CAR+SL-expressing T cells. PBMCs were transfected with the CSSP1, and GFP as a control. 24h later the transfected PBMCs were exposed to TMR-HER2 + SL CAR-T cells previously transduced with lentiviral vectors to express the TMR-HER2 CAR, with the specific ligand (SL) for the SCLs. The data suggests that the stimulation of the TMR-HER2+SL CAR with CSSP1 can enhance CAR-T cell proliferation. n=2 +/-SD.

Fig. 4: CSSP1 enhances CAR-T proliferation

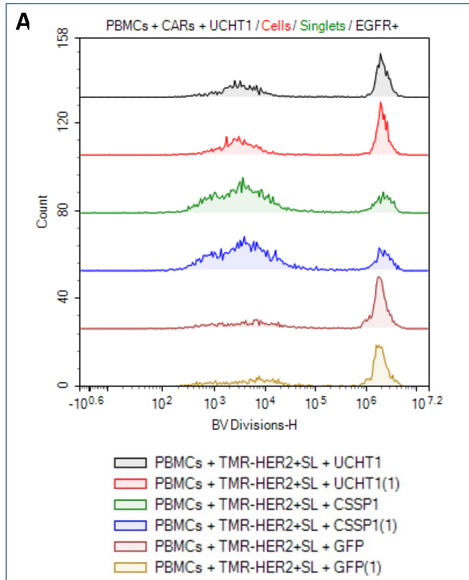

Figure 4: CSSP1 triggers activation and division of the TMR-HER2+SL CAR-T cells. (A) PBMCs were transfected with the CSSP1, and GFP as a control. 24h later the transfected PBMCs were exposed to TMR-HER2+SL CAR-T cells previously transduced with lentiviral vectors to express the TMR-HER2 CAR, with the specific ligand (SL) for the CSSPs. The CAR-T cells were loaded with Celltrace Violet (Thermo) to follow the divisions. Whereas UCHT1 (500 ng/mL) could trigger divisions, CSSP1 appeared more effective, and little to no divisions were observed when PBMCs were transfected with GFP, at D2. n=2 +/-SD.**(B)** Whereas the division ratio is ~1 for UCHT1 and GFP, it is higher than 4 for CSSP1-based stimulation, confirming the selectivity of our stimulation by CSSP1.

Fig. 5: CSSP1 functionally activates CAR-T cells

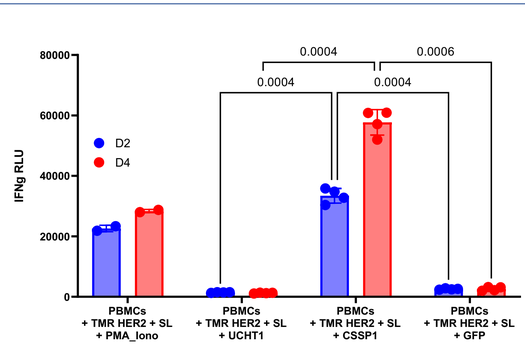

Figure 5: IFNg production by CSSP1-stimulated CAR-T cells. Supernatants from the experiment depicted in Figure 4 were collected at D2 and D4 post exposure to the CSSP1-expressing PBMCs, and an IFNγ ELISA performed. PMA/Ionomycin (1X, Biolegend) was used as a control. We observed that exposure of the TMR-HER2+SL CAR-T cells to CSSP1-expressing PBMCs resulted in the production of large amounts of IFNγ. n=2 +/-SD.

Conclusions

- mRNA encoding a CAR-specific stimulating protein (CSSP1) expressed on monocytes specifically recognizes a synthetic ligand engineered on to the TMR-HER2 CAR construct
- CSSP1 can induce NFAT-activation of TMR HER2+SL CAR T cells at a normal pH
- CSSP1 increases the ex-vivo proliferation of TMR HER2+SL CAR T cells
- CSSP1 enhances TMR HER2+SL CAR T cell division and proliferation
- CSSP1 induces functional activation of TMR HER2+SL CAR T cells in IFN-γ co-culture assays

References

1. Reinhard et al., Science 367, 446–453 (2020)
2. Moradian et al., Scientific Reports, (2020) 10:4181

Contact: Sid Kerkar; skerkar@exumabio.com