Poster nr. 273

Chimeric PGC1α expression in CAR-T cells improves metabolic function and anti-tumor efficacy in solid tumors

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Abstract

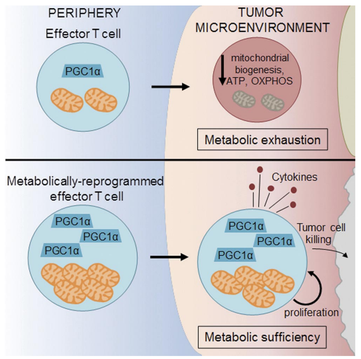
Background: The solid tumor microenvironment (TME) suppresses CAR T cell function through various mechanisms including competition for nutrients and chronic stimulation resulting in limited T cell effector functions or T cell exhaustion. Therefore, CAR T cells with enhanced metabolic fitness or more durable early memory phenotype could improve the clinical outcome against solid tumors. PPAR gamma coactivator 1α (PGC1α) is a transcriptional coactivator that influences many aspects of cellular metabolism. Previous studies have indicated that exogenous expression of PGC1α can enhance T cell anti-tumor activity, however the large size of PGC1α renders it difficult to co-express with a CAR. An N-terminal truncated PGC1α (NT-PGC1 α) has previously been shown to improve co-expression with a CAR, however, despite imparting mitochondrial benefits, NT-PGC1α failed to increase anti-tumor activity.

Methods: We developed a novel chimeric PGC1α consisting of an N-terminal truncation with the addition of an exogenous DNA binding domain derived from PPARg The chimeric PGC1α was co-expressed with a ROR1-targeted CAR in human T cells and was compared to CAR alone in in vitro and in vivo studies.

Results: We observed a decrease in the number of dysfunctional mitochondria and a subsequent increase in glucose uptake in CAR T cells expressing the chimeric PGC1α compared to CAR alone. These cells also demonstrated enhanced resistance to chronic antigen stimulation and a less differentiated and less exhausted phenotype compared to CAR alone. Moreover, when tested in vivo, CAR T cells expressing the chimeric PGC1α had superior anti-tumor activity compared to CAR T alone in a solid tumor model of clear cell renal cell carcinoma.

Conclusions: These data suggest that incorporation of this novel chimeric PGC1α in CAR T cells may be a promising approach to enhance CAR T cell efficacy in patients with solid tumors.

Introduction



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- PGC1a is master transcriptional regulator of mitochondrial genes
- Overexpression has been shown to increase mitochondrial biogenesis and function
- Advantageous for T cells in immuno-suppressive environments
- Very large protein >700 residues
- Engineering effort to reduce size to a smaller functional entity ~300 residues

chPGC1a Improves CAR T Metabolic Function

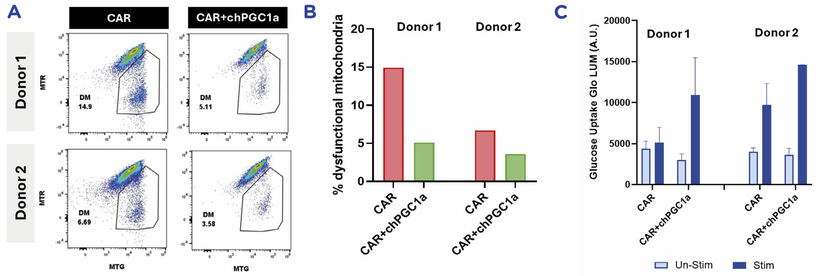


Figure 2. chPGC1a reduces the number of dysfunctional mitochondria and improves glucose uptake. (A and B) Dysfunctional mitochondria present in CAR or CAR+chPGC1a T cells were quantified using flow cytometry and defined as MitoTracker Red low/negative and MitoTracker Green positive. **(C)** Respective transduced cells were stimulated with ROR1 antigen for 72 h and glucose and uptake was measured in a luminescence-based assay and reported as background-subtracted arbitrary luminescence units (A.U.).

TN+SCM Cell Subset Enriched in chPGC1a CAR T Cells

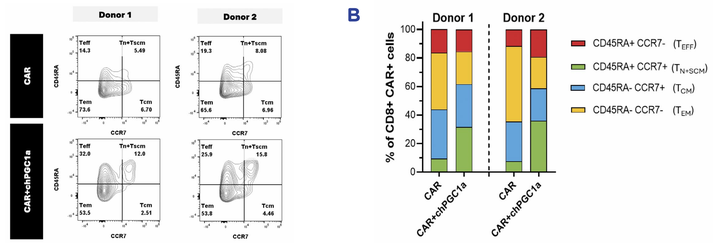


Figure 3. Expression of chPGC1a polypeptide increased the TN+SCM Cell subset. T cell memory subsets were analyzed in CAR T or CAR+chPGC1a Tcells by flow cytometry. **(A)**. Representative flow cytogram indicates the gating strategy used in analyzing the T cell memory subsets. **(B)** Tabulated flow cytometric data gated on CD8+ CAR+ T cells. Results shown here are from two independent donors. Gating strategy – Effector T cell (T_{EFF}): CD3+ CD8+ CAR+ CD45RA+ CCR7-; Naïve and Stem Cell Memory (T_{N+SCM}): CD3+ CD8+ CAR+ CD45RA+ CCR7+; Central Memory T cell (T_{CM}): CD3+ CD8+ CAR+ CD45RA- CCR7+; Effector Memory T cell (T_{EM}): CD3+ CD8+ CAR+ CD45RA- CCR7-.

Co-Expression of CAR + chPGC1a

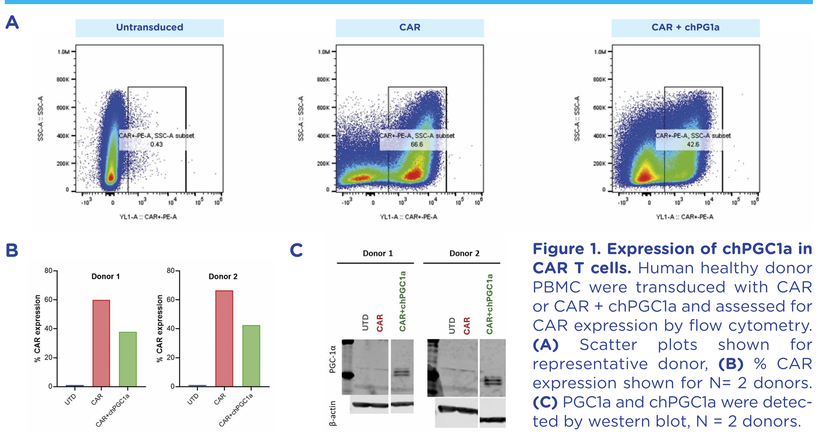


Figure 1. Expression of chPGC1a in CAR T cells. Human healthy donor PBMC were transduced with CAR or CAR + chPGC1a and assessed for CAR expression by flow cytometry. **(A)** Scatter plots shown for representative donor, **(B)** % CAR expression shown for N= 2 donors. **(C)** PGC1a and chPGC1a were detected by western blot, N = 2 donors.

T_{N+SCM} Cell Subset is Further Enriched Following Antigen Stimulation

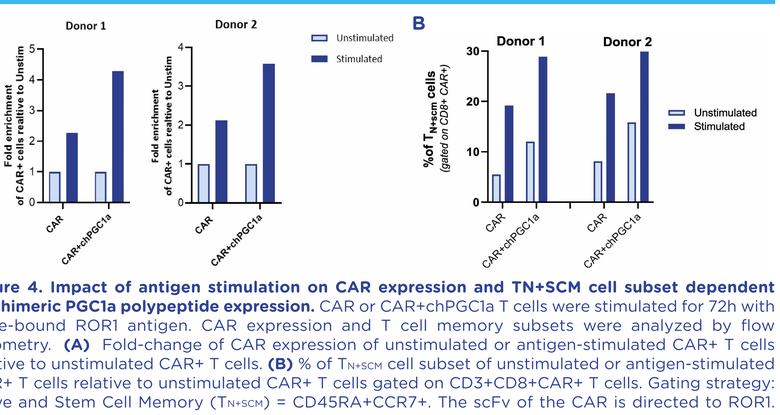
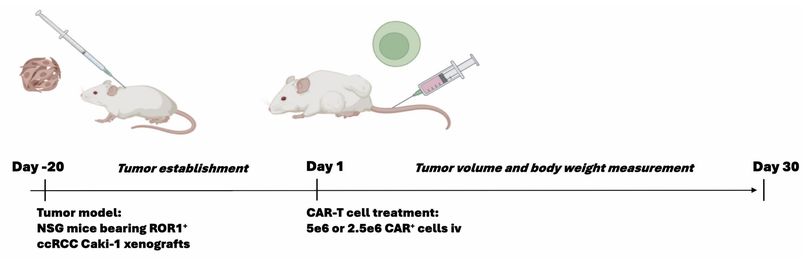


Figure 4. Impact of antigen stimulation on CAR expression and TN+SCM cell subset dependent on chimeric PGC1a polypeptide expression. CAR or CAR+chPGC1a T cells were stimulated for 72h with plate-bound ROR1 antigen. CAR expression and T cell memory subsets were analyzed by flow cytometry. **(A)** Fold-change of CAR expression of unstimulated or antigen-stimulated CAR+ T cells relative to unstimulated CAR+ T cells. **(B)** % of T_{N+SCM} cell subset of unstimulated or antigen-stimulated CAR+ T cells relative to unstimulated CAR+ T cells gated on CD3+CD8+CAR+ T cells. Gating strategy: Naïve and Stem Cell Memory (T_{N+SCM}) = CD45RA+CCR7+. The scFv of the CAR is directed to ROR1. Results shown here are from two independent donors.

Co-expression with chPGC1a Improves Anti-Tumor Efficacy



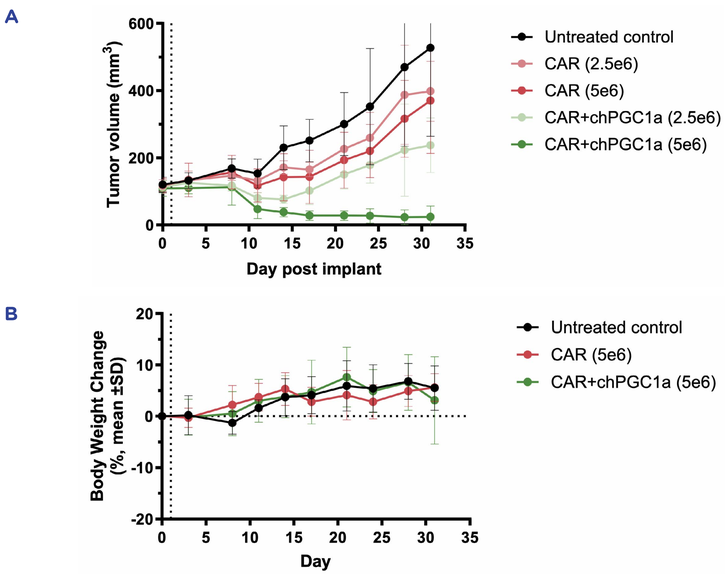


Figure 5. In vivo anti-tumor efficacy in CAKI-1 (Human Renal Cell Carcinoma) xenograft mouse model. NOD-SCID-IL2Rγ null (NSG) mice having established CAKI-1 (human clear cell renal cell carcinoma) tumors were randomized into treatment groups based on tumor volume (117 ±25 mm^3 on day 20 post implantation). Animals received a single intravenous administration of ROR1-targeted CAR-Ts on Day 21 post tumor implantation (study day 1) at 5x 10^6 or 2.5x10^6 CAR+ T cells. Control animals were left untreated. Tumor volume and body weights were monitored twice weekly until Day 30. Mice were euthanized when tumor volumes reached 1000 mm^3, or in the event of tumor ulceration. **(A)** Plot shows mean tumor volume of tumor-bearing mice **(B)** Plot shows in graph body weight change in % in untreated and treated groups receiving 5x 10^6 CAR+ T cells (No change in bodyweights for 2.5x10^6 dose, data not shown). (N=5 mice per group)

Summary:

- **Expression of a chimeric PGC1a transgene in CAR T cells have fewer dysfunctional mitochondria and improved glucose uptake compared to CAR T cell controls**
- **CAR+chPGC1a T cells have more T_{N+SCM}, and this less-differentiated phenotype is enriched following antigen stimulation.**
- **chPGC1a enhances CAR T anti-tumor efficacy with no overt signs of toxicity.**
- **These data suggest that co-expression CAR + chPGC1a is a promising approach to enhancing CAR T cell efficacy in solid tumors.**

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