様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Metabolic reprogramming of antitumor T cells for optimal adoptive immunotherapy

研究課題名（英文）Metabolic reprogramming of antitumor T cells for optimal adoptive immunotherapy

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研究成果の概要（和文）：選択的AMP活性化プロテインキナーゼ（AMPK）阻害剤であるドルソモルフィンの投与により、CAR-T細胞の未分化メモリー形質の維持が促進され、かつサイトカイン分泌能も高まることがわかった。また同剤投与により、T細胞の増殖能には影響がなかった。しかし、それは細胞傷害性と腫瘍の成長抑制に付加的な影響を及ぼしませんでした。そのうえ、それは刺激の数回の後で、T細胞消耗をほとんど抑えることができません。T細胞機能的な影響のAMPK経路の役割のメカニズムは、現在の研究の下でさらに調査される必要があります。

研究成果の学術的意義や社会的意義
キメラ抗原受容体導入T細胞療法がB細胞性腫瘍において高い臨床効果を示したことから注目されている。しかし他のがんに対しては十分な臨床効果が得られておらず、改良が必要である。不十分な治療効果の主な要因として、持続的な抗原刺激に伴いT細胞の機能が低下し、かつ分化に伴い長期生存能が低下することが挙げられる。本研究には、T細胞機能と密接に関わる代謝関連分子に着目し、同分子を標的として外的に修飾することによりT細胞機能を高めることを目指す。本研究で得られた成果は、これまで十分な治療効果が得られていない固形腫瘍を中心とした難治がんに対する養子免疫療法の治療効果を高めるために応用することを将来的な目標とする。

研究成果の概要（英文）：CAR-T cells treated with dorsomorphin, a selective AMP-activated protein kinase (AMPK) inhibitor, significantly maintained a young memory phenotype and prolonged CAR-T cell persistence in mice without affecting proliferative capacity. But it had no additive effect on cell cytotoxicity and tumor growth inhibition. In addition, it can hardly suppress T cell exhaustion after several times of stimulation. The mechanism of the role of the AMPK pathway in T cell functional effect needs to be investigated further under current research.

研究分野：腫瘍免疫応答

キーワード：adoptive immunotherapy　CAR-T　AMPK　dorsomorphin

１．研究開始当初の背景

The significance of metabolism in antitumor T cell immunity has become apparent. However, it has yet to be known how exogenous manipulation can modulate T cell functions. We have explored metabolic targets associated with T cell functions and identified that Reagent A, an inhibitor of the critical metabolic pathway, helped maintain CD8+ T cells with a young memory phenotype and cytokine polyfunctionality with repeated antigen exposures. Based on this data, we hypothesize that inhibiting the metabolic pathway in T cells can enhance their durable effector functions and survival capacity.

２．研究の目的

The efficacy of adoptive immunotherapy against solid tumors has been limited. One of the reasons underlying the insufficient response is that antitumor T cells exposed to persistent antigenic stimulation undergo exhaustion. A proportion of exhausted T cells with an undifferentiated memory phenotype are more sensitive to immune checkpoint inhibitors and can persist longer than terminally exhausted T cells. Our research focuses on exogenous manipulation of metabolic profiles, which may be able to support the maintenance of a progenitor memory phenotype in T cells, resulting in durable therapeutic efficacy in the solid tumor. Successful completion of this study will improve the therapeutic efficacy of adoptive T cell immunotherapy against currently refractory cancer.

３．研究の方法

We thoroughly studied metabolic targets associated with T cell differentiation and effector functions in the context of repetitive antigenic stimulation by treating CAR-T cells with an array of specific inhibitors. We have identified that the CAR-T cells treated with dorsomorphin, a selective AMP-activated protein kinase (AMPK) inhibitor, significantly maintained a young memory phenotype and cytokine polyfunctionality without affecting proliferative capacity.

Although it is generally considered that the AMPK is required for effector T cell functions, our data suggest that inhibiting AMPK may have a beneficial influence on antitumor immunity. To test this notion, we performed the following experiments.

We injected NSG mice with dorsomorphin-treated or untreated T cells and monitored theirs *in vivo* persistence. We also tested the antitumor efficacy of dorsomorphin-treated CAR-T cells in the solid tumor model. NSG mice were subcutaneously transplanted with the A375 melanoma cell line stably transduced with mesothelin and then treated with anti-mesothelin CAR-T cells. Mice were regularly injected with dorsomorphin or vehicle and analyzed for tumor progression. We also examined the tumor-infiltrating CAR-T cell functions *ex vivo*; intratumoral CAR-T cells were isolated and cocultured with the A375-mesothelin to measure cytokine secretion. In addition to pharmacologic inhibition, we also tested the effect of mutagenesis and genetic knockout of AMPK subunit(s) in CAR-T cells. We performed qPCR analysis to elucidate how the AMPK inhibition affects the transcriptional profiles of CAR-T cells.

４．研究成果

We have identified that the CAR-T cells treated with dorsomorphin (DM), a selective AMP-activated protein kinase (AMPK) inhibitor, significantly maintained a young memory phenotype and cytokine polyfunctionality without affecting proliferative capacity (Figure 1). The AMPK is one of the essential components regulating T cell metabolism and related to memory T cell formation and effector T cell differentiation.

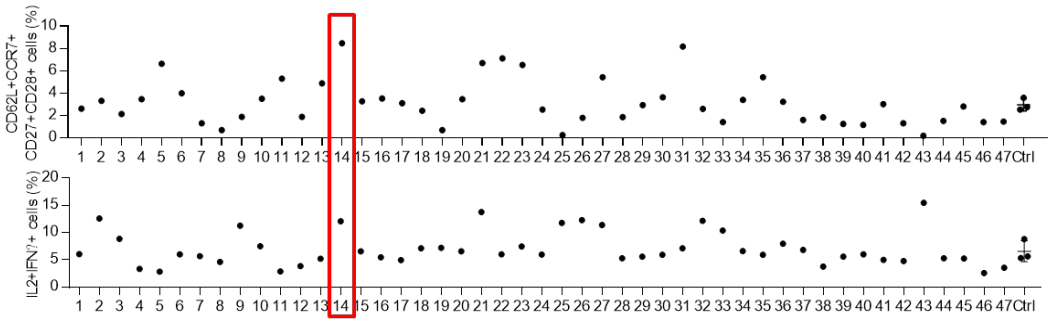

Figure 1. Anti-CD19 CAR-T cells were repeatedly stimulated by the antigen in the presence of individual inhibitors (#1-47). Memory T cell phenotypes (upper panel) and cytokine production (lower panel) were analyzed after the 3rd stimulation (#14 denotes Dorsomorphin).

We added DM prior to restimulation (Figure2A) and confirmed that CAR-T cells treated with DM could maintain younger memory makers after stimulation compared to the control group. (Figure 2B,C)

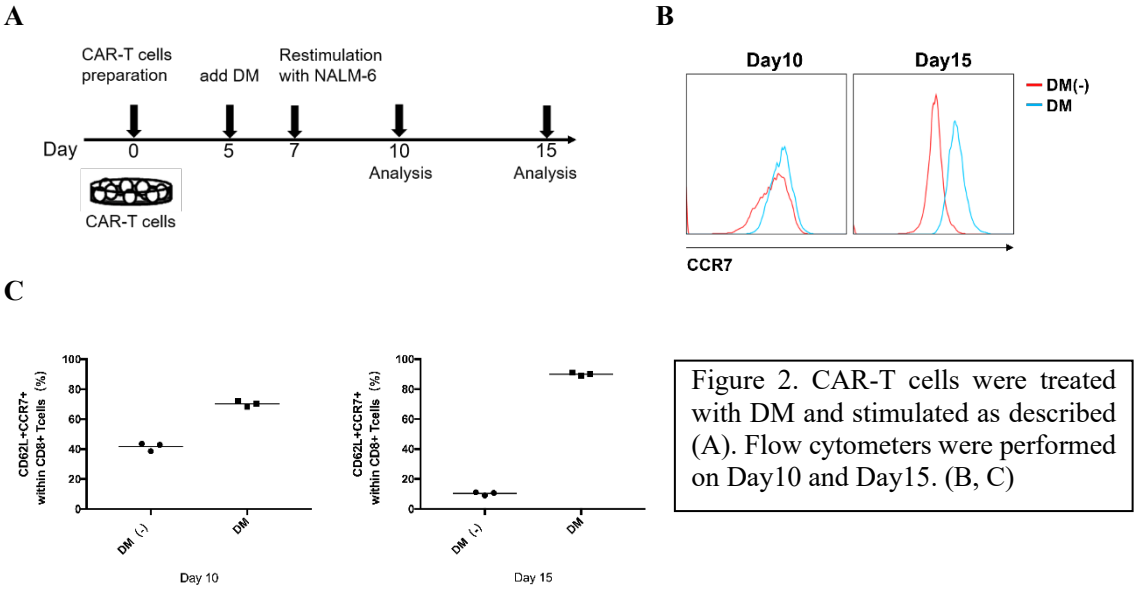

Figure 2. CAR-T cells were treated with DM and stimulated as described (A). Flow cytometers were performed on Day10 and Day15. (B, C)

Identical results were collected in vivo that DM-treated T cells had a longer persistence in peripheral blood than the control group. (Figure 3A, B)

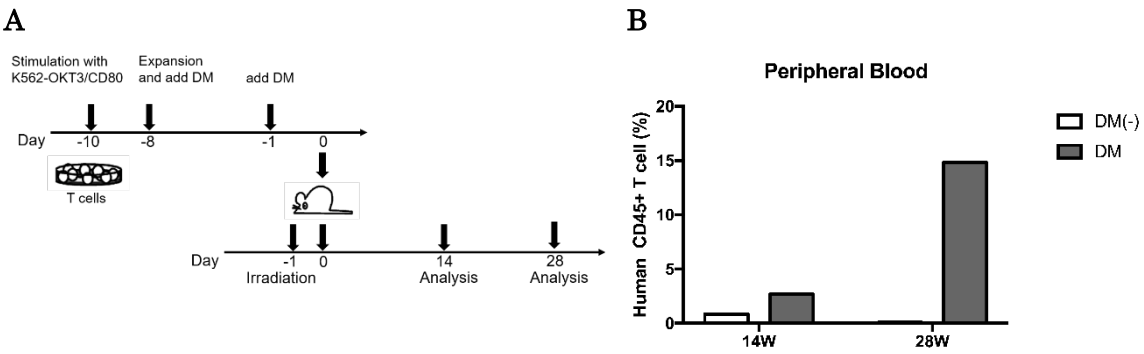

Figure 3. T cells were stimulated and treated with DM before infusion into mice. (A) After infusion, peripheral blood was collected on Day14 and Day28. Results were evaluated by Flow cytometers. (B)

Next, we evaluated Cytotoxicity in vitro and the anti-tumor effect in vivo. We used 14g2a CAR-T cells to coculture with Nalm6-GD2sGD3s cells. After 24h coculturing, we accessed the cytotoxicity by Flow cytometers. CAR-T cells treated with DM showed good cytotoxicity on tumor cells but did not have an enhanced effect on the DM-untreated group. (Figure 4A) Meanwhile, in vivo mice models, DM-treated CAR-T cells had no additive effect on tumor suppression after infusion into mice. (Figure 4B)

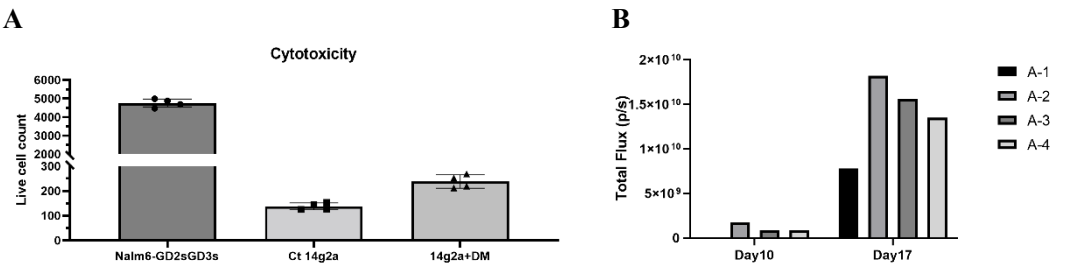

Figure 4. Nalm6-GD2sGD3s were cocultured with 14g2a CAR-T cells treated or untreated with DM. Cytotoxicity was evaluated after 24h incubation. (A) Nalm6-GD2sGD3s were infused into Mice (Group A) on Day0 and DM-treated CAR-T cells were injected on Day7. Tumor volume was evaluated on Day10 and Day17 respectively. (B)

Meanwhile, we collected CAR-T cells from peripheral blood and stimulated them in vitro to evaluate cytokine secretion. (Figure5A) DM-treated CAR-T cells did not significantly affect Cytokine secretion, and similar results were obtained in dominate-negative PRKAA1 CAR-T cells in vitro. (Figure5B)

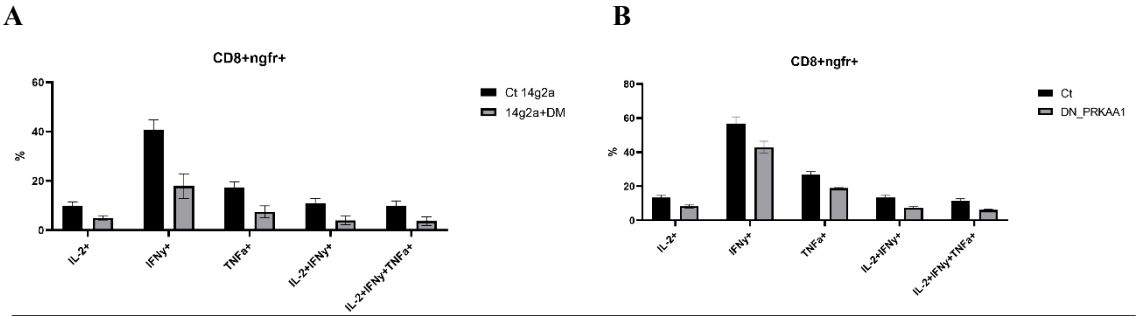

Figure 5. 14g2a CAR-T cells were collected from PB and cytokine was evaluated after stimulation in vitro. (A) Cytokine secretion of DN-PRKAA1 CAR-T cells were evaluated in vitro. (B)

To evaluate the effect on T cell exhaustion, we stimulated 14g2a CAR-T cells several times to lead T cells into exhaustion status. We defined T cell exhaustion by exhaustion surface markers such as PD1, LAG3, and TIM3. DM-treated CAR-T cells showed a mild effect on T cell exhaustion after one-time stimulation. (Figure6A) But it had no additive effect on T cell exhaustion after 3-time stimulation. (Figure6B)

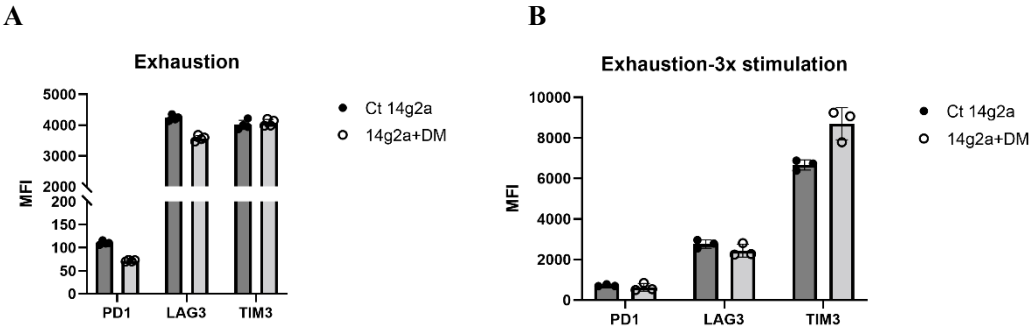

Figure 6. 14g2a CAR-T cells were stimulated for one-time (A) and 3-time (B). Exhaustion markers were evaluated respectively in vitro.

Finally, we performed qPCR to evaluate the transcription factors changes after DM treatment on CAR-T cells and compared them with control. Interestingly, the result showed a difference from what we observed above. DM-treated CAR-T cells had no additive-enhanced effect on memory markers except IL7R. And it had effective suppression on exhaustion markers. (Figure7)

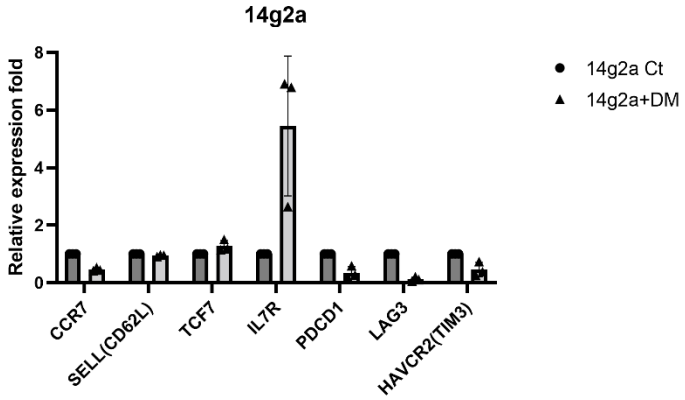

Figure 7. qPCR was performed after 14g2a CAR-T cells were stimulated and treated with or without DM. CCR7, SELL, TCF7 and IL7R were represented as memory markers and PDCD1, LAG3 and HAVCR2 were represented as exhaustion markers.

After considering the results above, we can make a conclusion that CAR-T cells treated with dorsomorphin, a selective AMP-activated protein kinase (AMPK) inhibitor, significantly maintained a young memory phenotype and prolonged CAR-T cell persistence in mice without affecting proliferative capacity. But it had no additive effect on cell cytotoxicity and tumor growth inhibition. In addition, it can hardly suppress T cell exhaustion after several times of stimulation. The mechanism of the role of the AMPK pathway in T cell functional effect needs to be investigated further under current research.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関