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研究課題名（和文）Application of gold-catalyzed hydroamination in sialic acids for the cancer-localized in vivo release of an immunotherapy drug

研究課題名（英文）pplication of gold-catalyzed hydroamination in sialic acids for the cancer-localized in vivo release of an immunotherapy drug

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研究成果の概要（和文）：細胞内のシアル酸転移酵素の阻害は、新たながん治療戦略として期待されますが、既存の阻害剤は正常組織にも作用してしまうため、腎臓の機能障害などの副作用を引き起こすことが課題となっていました。本研究は、がん細胞内でのみ阻害剤を合成し作用させれば副作用の恐れが少ないと考えました。そこで、アジド基を持つ化合物とアクロレインが選択的に環化付加反応を起こすことを利用した、阻害剤の原料となるプロドラッグを設計しました。プロドラッグががん内でアクロレインと反応して、ドラッグ活性体が合成され、シアル酸転移酵素を阻害し、がんを治療することができました。副作用である腎臓の機能障害による体重増減は見られませんでした。

研究成果の学術的意義や社会的意義
本研究では、アクロレインが多く存在するがん細胞内でシアル酸転移酵素阻害剤を合成し、がん細胞上のみで糖鎖構造を合成化学的に変化させ、副作用なくマウスのがんを治療することに成功しました。がん細胞内で薬剤を合成するという本研究の戦略は、他の作用機序を有する抗がん剤の開発においても、副作用を減らす戦略としての活用が期待されます。

研究成果の概要（英文）：Abnormal glycosylation is a hallmark of cancer, and hypersialylation increases tumor metastasis by promoting immune evasion and inducing tumor cell invasion and migration. Inhibiting sialylation is thus a potential anticancer treatment strategy. However, targeting sialic acids is difficult because of the lack of selective delivery tools. Here, we present a prodrug strategy for selectively releasing the global inhibitor of sialylation peracetylated 3Fax-Neu5Ac (PFN) in cancer cells using the reaction between phenyl azide and endogenous acrolein, which is overproduced in most cancer cells. The prodrug significantly suppressed tumor growth in mice as effectively as PFN without causing kidney dysfunction, which is associated with PFN. The use of sialylated glycans as immune checkpoints is gaining increasing attention, and the proposed method for precisely targeting aberrant sialylation provides a novel avenue for expanding current cancer treatments.

研究分野：Chemical biology

キーワード：Prodrug　Sialyltransferases　In vivo synthesis　Acrolein

１．研究開始当初の背景

One of the most remarkable changes in cancer glycosylation is aberrant sialylation due to the marked upregulation of sialyltransferases, which catalyze the addition of sialic acid to growing glycochains on the cell surface to form sialoglycans. Desialylation of cancer cells potentiates NK cell–mediated cytotoxicity and promotes the clearance of cancer cells injected into mice, suggesting that targeting aberrant sialylation could be developed as an effective anticancer treatment.

２．研究の目的

A cell-permeable peracetylated $3F_{ax}$-Neu5Ac (PFN) effectively inhibited all sialyltransferases *via* a mechanism involving its intracellular conversion to an active inhibitor, CMP-$3F_{ax}$-Neu5Ac, thereby reducing overall sialylation in cultured cells. However, despite the efficacy of PFN in decreasing sialylated glycans in most tissues in the murine model, it causes kidney dysfunction because of the depletion of sialic acids from podocytes, which impairs glomerular filtration.

３．研究の方法

Here, we present a prodrug strategy for selectively releasing the global inhibitor of sialylation peracetylated $3F_{ax}$-Neu5Ac (PFN) in cancer cells using the reaction between phenyl azide and endogenous acrolein, which is overproduced in most cancer cells. As shown in Figure 1, the 2,6-diisopropylphenyl azide moiety of **1** selectively reacted with cancer cell–endogenous acrolein to release C2-hydroxy PFN **2**, which was converted to CMP-$3F_{ax}$-Neu5Ac to inhibit sialyltransferases, thereby enhancing cancer immunotherapy and avoid unwanted side effects.

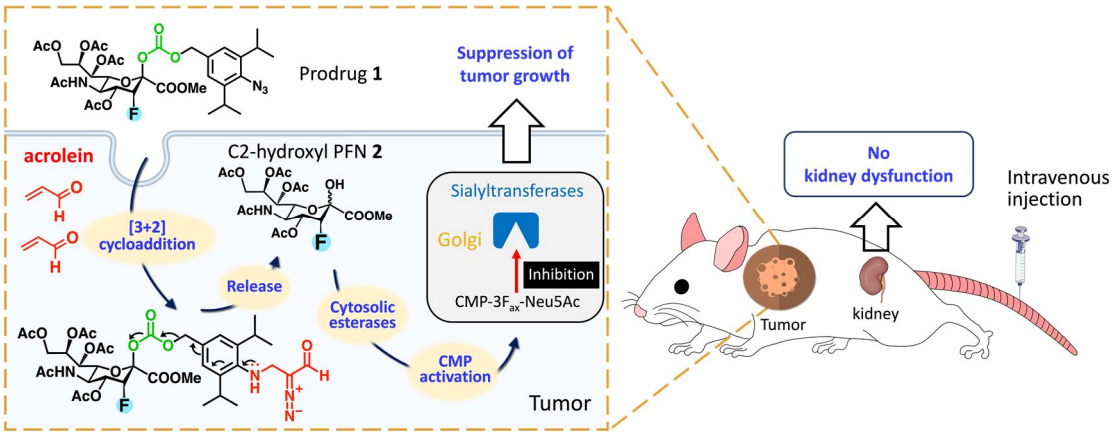

Figure 1. Schematic of the PFN-based prodrug **1** strategy for the selective release of C2-hydroxyl PFN **2** in tumor sites *in vivo* through endogenous acrolein to enhance cancer immunotherapy while avoiding kidney dysfunction caused by PFN.

４．研究成果

As reported by Paulson *et al.*, mice treated with 300 mg/kg of PFN develop kidney dysfunction as an adverse effect. According to the cell-based results, in the last stage of this study, we investigated the efficacy of prodrug **1** in the treatment of subcutaneous B16F10-xenografted mice and examined whether **1** could be used to avoid the kidney dysfunction caused by PFN. Because of the limited solubility of **1** in saline solution, mice were divided into three groups as follows: Vehicle, PFN (60 mg/kg), and prodrug **1** (60 mg/kg) *via* intravenous administration every day for 5 consecutive days for a treatment total of 300 mg/kg. The control group received a saline solution (vehicle) to replace the compounds in the treatment protocol. Tumor size was monitored for 16 days. The rate of tumor growth significantly decreased in the PFN (Fig. 2B, blue line) and prodrug **1** (Fig. 2B, red line) groups compared with the vehicle group (Fig. 2B, orange line). Mice were sacrificed on day 16 and tumors were extracted (Fig. 2C). The findings shown in Fig. 2B–C demonstrate

that PFN and prodrug **1** significantly suppressed tumor growth compared with the control condition (vehicle) by inhibiting sialyation in tumors to enhance the immunotherapy effect.

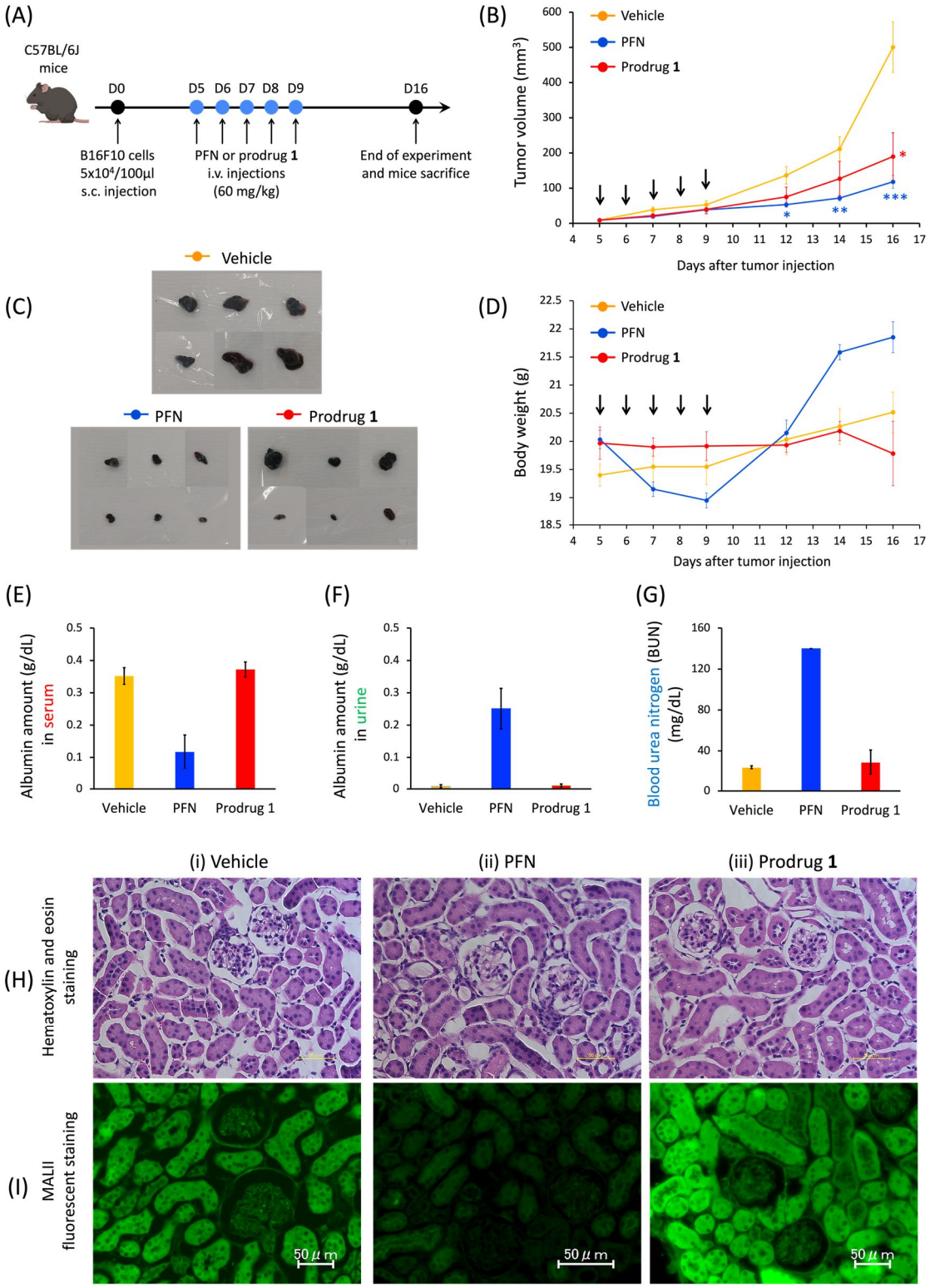

Figure 2. *In vivo* prodrug **1** activation by acrolein against B16F10 tumor growth in mice. (A) Schematic representation of the *in vivo* experiment. B16F10 cell xenograft–bearing C57B/6J mice were treated with vehicle, PFN, or prodrug **1**. Tumors were initially implanted in mice and developed over 5 days before therapy. When tumor sizes reached 15-20 mm^3, the B16F10 tumor-bearing mice were randomly divided into 3 groups: vehicle group (n = 6); PFN treatment (n = 6); prodrug **1** treatment (n = 6). A dose of 60 mg/kg was administered in the form of daily injections for 5 days through intravenous injection. The tumor volume and body weight of the mice were recorded until day 16 post-injection. Tumor volume was quantified using an equation of $V = W^2 \times L \times 0.4$, where W and L represented the minor and major length of the tumor, respectively. On day 16 post-injection, mice were sacrificed, urine and blood were collected, and their tumors were excised, imaged, and weighed. (B) Measurement of tumor size (mm^3) in mice over

time. (C) Visual comparison of extracted tumors shows the extent of growth inhibition at 11 days after the start of therapy (n = 6). (D) Body weight changes in the different groups of mice. Determination of albumin levels in serum (E) or urine (F) pooled from mice at 11 days after the start of therapy. (G) Determination of blood urea nitrogen (BUN) in blood pooled from mice at 11 days after the start of therapy. (H) Hematoxylin and eosin staining on kidney tissue in various groups of mice at 11 days after the start of therapy. (I) Fluorescence staining by MALII lectin on kidney tissue in various groups of mice at 11 days after the start of therapy. Black arrows under the horizontal axis indicate the day of treatment with the compounds. Data in (B and D-G) are presented as the mean ± SE, n = 6 biological replicates. P values were determined using a two-tailed Student's *t*-test. *P < 0.05, **P < 0.01, ***P < 0.001 compared to the vehicle group.

PFN causes kidney dysfunction in mice, which manifests as edema, weight gain, excretion of albumin in the urine, and albumin loss from the blood. In this study, mice receiving PFN showed higher body weight (Fig. 2D, blue line) than those in the vehicle group (Fig. 2D, orange line) at approximately 8 days after the start of treatment, indicating the occurrence of edema. Analysis of the blood and urine in the three groups showed a decrease in blood albumin (Fig. 2E) concomitant with an increase in urine albumin (Fig. 2F) in the PFN group compared with the vehicle group, with a substantial increase in urea nitrogen in the blood (BUN) (Fig. 2G), indicating that PFN caused kidney dysfunction in mice. By contrast, in the prodrug **1** group, these measurements were comparable to those in the vehicle group. In addition, histochemical studies (Fig. 2H-I) were performed on day 16 paraffin-embedded kidney tissue sections from mice treated with vehicle, PFN, and prodrug **1**. Although the hematoxylin and eosin staining on the kidney tissues of treated mice in the three groups did not show histological changes (Fig. 2H), the fluorescent lectin staining of these kidney tissues revealed that the PFN group (Fig. 2I-ii) obviously decreased MALII staining relative to the vehicle and prodrug **1** groups (Fig. 2I-i and iii). The data in Fig. 2I demonstrated that prodrug **1** did not alter histochemical characteristics or sialic acid expression in the kidney tissues of mice. These *in vivo* results of Fig. 2 indicate that activation of prodrug **1** by endogenous acrolein in tumors to release 2-hydroxyl of PFN **2** for inhibiting sialic acid formation may enhance tumor immunotherapy to inhibit tumor growth without causing kidney dysfunction, highlighting the potential of this strategy for future applications in cancer therapy. To apply the prodrug strategy to clinical trial testing in the future, we will proceed with a preclinical study using a patient-derived xenograft (PDX) mouse model to further investigate the effect of prodrug **1** on tumor therapy and evaluate its toxicity.

This study represents a significant advance in research into targeting aberrant sialylation in cancer to enhance cancer immunotherapy. Cell-based experiments showed that endogenous acrolein can be used to activate prodrug **1** to release **2**, thereby inhibiting sialylation in cancer cells to increase susceptibility to NK cell–mediated cytolysis. Prodrug **1** significantly suppressed B16F10 tumor growth in mice as effectively as PFN without causing kidney dysfunction. Because the use of sialylated glycans as immune checkpoints is gaining increased attention,[33] this system for precisely targeting aberrant sialylation offers another avenue for expanding current cancer immunotherapy.

The paper presenting these findings has been finalized and published (*Chem. Sci.* **2024**, DOI: 10.1039/D4SC00969J)

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　0件／うちオープンアクセス　1件）

１．著者名 Kasahara Takatsugu、Chang Tsung-Che、Yoshioka Hiromasa、Urano Sayaka、Egawa Yasuko、Inoue Michiko、Tahara Tsuyoshi、Morimoto Koji、Pradipta Ambara R.、Tanaka Katsunori	４．巻 -
２．論文標題 Anticancer approach by targeted activation of a global inhibitor of sialyltransferases with acrolein	５．発行年 2024年
３．雑誌名 Chemical Science	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D4SC00969J	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔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