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研究課題名（和文）Application of gold-catalyzed glycosylation under aqueous condition for the tumor-localized in vivo synthesis of anticancer drugs

研究課題名（英文）Application of gold-catalyzed glycosylation under aqueous condition for the tumor-localized in vivo synthesis of anticancer drugs

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研究成果の概要（和文）：今回の研究では、金触媒によって薬剤の骨格を生体内で構築するという新たなプロドラッグ戦略を開発しました。従来のプロドラッグ戦略は、保護基を導入できる薬剤にしか適用できませんでした。一方で、今回開発した手法では保護基を導入する官能基がなくても、薬剤の骨格を直接構築することで薬効を制御できるため、プロドラッグ戦略の適用範囲を広げることができます。さらに、生体内に導入可能な人工金属酵素によっても、このプロドラッグの活性化が行えることが本研究で示されました。今後、生体内の標的細胞に輸送された人工金属酵素で今回の化学反応を行うことができれば、生体内のがん細胞で薬剤を合成することも可能になると考えられます。

研究成果の学術的意義や社会的意義
This is fundamental research that aims to produce new systems and reactions that could one day be exploited to develop new targeted medicinal therapies. The findings of the study were distributed to the scientific community through peer-reviewed journals and conferences.

研究成果の概要（英文）：Chemotherapy is one of the many strategies for treating cancer. However, many chemotherapy drugs produce unwanted side effects because in addition to attacking cancer cells, they cause collateral damage to healthy cells. One strategy for minimizing side effects is to use a prodrug—an inactive compound that is converted into an active drug on undergoing a chemical reaction at the target site. The project introduce a strategy based on the gold-catalyzed a phenanthridinium-based prodrug via hydroamination in aqueous condition. To make the strategy biocompatible, a gold artificial metalloenzyme (ArM) based on human serum albumin, rather than the free gold catalyst, was used for prodrug activation. The albumin-based gold ArM protected the catalytic activity of the bound gold metal even in the presence of up to 1 mM glutathione. The drug synthesized via the gold ArM exerted a therapeutic effect in cell-based assays, highlighting the potential usefulness of the gold ArM in vivo applications

研究分野：Chemical Biology

キーワード：biocatalysis　biocompatibility　artificial metalloenzyme　gold catalysis　drug synthesis

１．研究開始当初の背景

Since the most current chemotherapeutic drugs lack specificity for cancer cells, resulting in causing systemic toxicity in cancer patients. A research trend has emerged centered on localized drug delivery to minimize side effects of chemotherapeutic drugs toward untargeted normal cells. Due to the bioorthogonality and outstanding catalytic activity of transition metals, application of them to catalyze uncaging reactions to release bioactive drugs have recently developed for anticancer approaches.

２．研究の目的

New strategies using abiotic metal as a trigger for prodrug design are still in high demand because current prodrug design relies heavily on the uncaging strategy (Fig 1A). As a result, only drugs that have amine or hydroxyl groups are suitable for this prodrug design. However, many drugs contain N-heterocyclic rings and polycyclic aromatic hydrocarbon structures. Hence, it would be greatly beneficial to develop a suitable prodrug strategy for such aromatic drugs.

３．研究の方法

Here, the project reported a new strategy for prodrug design using 2'-alkynyl-N-methyl-2-biphenylamine as framework for the prodrug. Using this method, it is possible to synthesize 5-methyl phenanthridinium derivatives via gold-catalyzed hydroamination (Fig 1B). The prodrug strategy not only can generate 5-methyl phenanthridinium derivatives to mimic naturally occurring alkaloids bearing a 5-methyl phenanthridinium core (i.e. sanguinarine), but can also synthesize quaternary ammonium cation compounds.

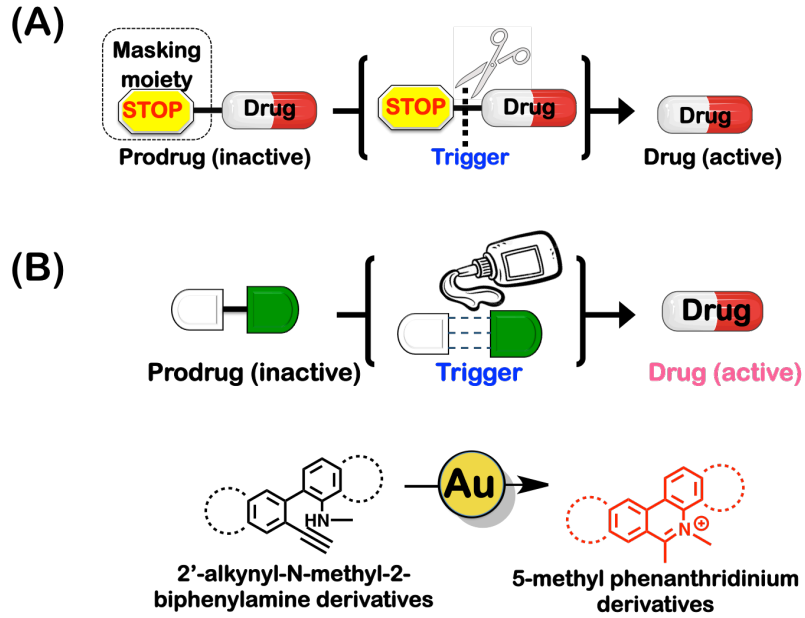

Figure 1. Strategy of prodrug design (a) General design of prodrugs based on drug-release strategy. (b) This work represents a new prodrug design based on a drug synthesis strategy for synthesis of 5-methyl phenanthridinium derivatives via gold-triggered 2'-alkynyl-N-methyl-2-biphenylamine derivatives hydroamination.

４．研究成果

According to the literature, the phenanthridinium compounds can exert cytotoxicity against various cancer cell lines through interacting with DNA, resulting in cell apoptosis. As depicted in Figure 2A, the

Au-b [Au(I) complex] exhibited excellent catalytic activity, achieving drug **2** at 81% yield from prodrug **1** in physiological condition.

Next, we carried out an experiment to determine the *in cellulo* activity of the prodrug against A549 cancer cells (Figure 2B). The results revealed that a mixture of prodrug **1** with an **Au-1** concentration of 2.5 μM induced significant decrease in cell growth relative to the individual effects of each compound at the corresponding concentrations. The data clearly showed that a prodrug strategy based on direct synthesis of drug **2** by gold-catalyzed hydroamination under mild conditions could be applied to cancer therapy.

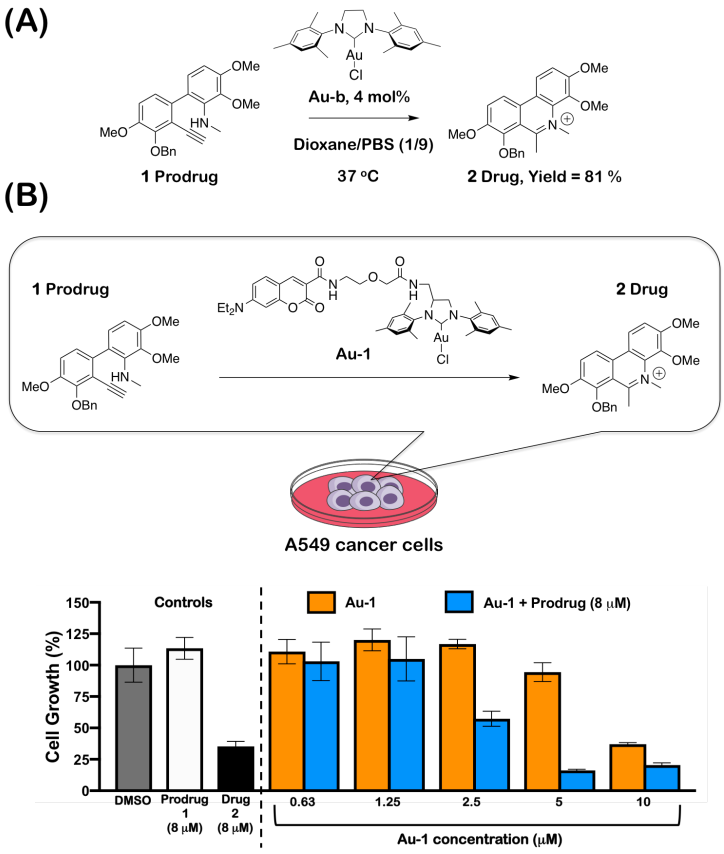

Figure 2. (A) Investigation of the cyclization of prodrug **1** in the presence of **Au-a** catalyst. (B) Cytotoxicity studies for prodrug activation via **Au-1** gold-catalysis.

Despite the promising results, practical application of the gold-mediated prodrug strategy in cancer treatment has been limited by a major obstacle, namely, biocompatibility. Glutathione (GSH), a significant cell metabolite in biological systems, is often present at concentrations in the range of 0.5–10 mM. Upon administering gold complexes to GSH, a ligand-exchange reaction with GSH can occur rapidly, abrogating the activity of gold complexes. To make the prodrug strategy biocompatible with cell metabolites, development of an albumin-based gold artificial metalloenzyme (ArM) as a trigger, rather than of the free gold metal complex, for the gold-mediated drug synthesis strategy could be an ideal manner (Fig 3A). We incorporated the **Au-1** into human serum albumin through the interaction of coumarin-based derivatives to the hydrophobic binding pocket of albumin to generate **albumin–Au-1**. Thereby, albumin can prevent hydrophilic GSH from entering the cavity to destroy the bound gold catalyst.

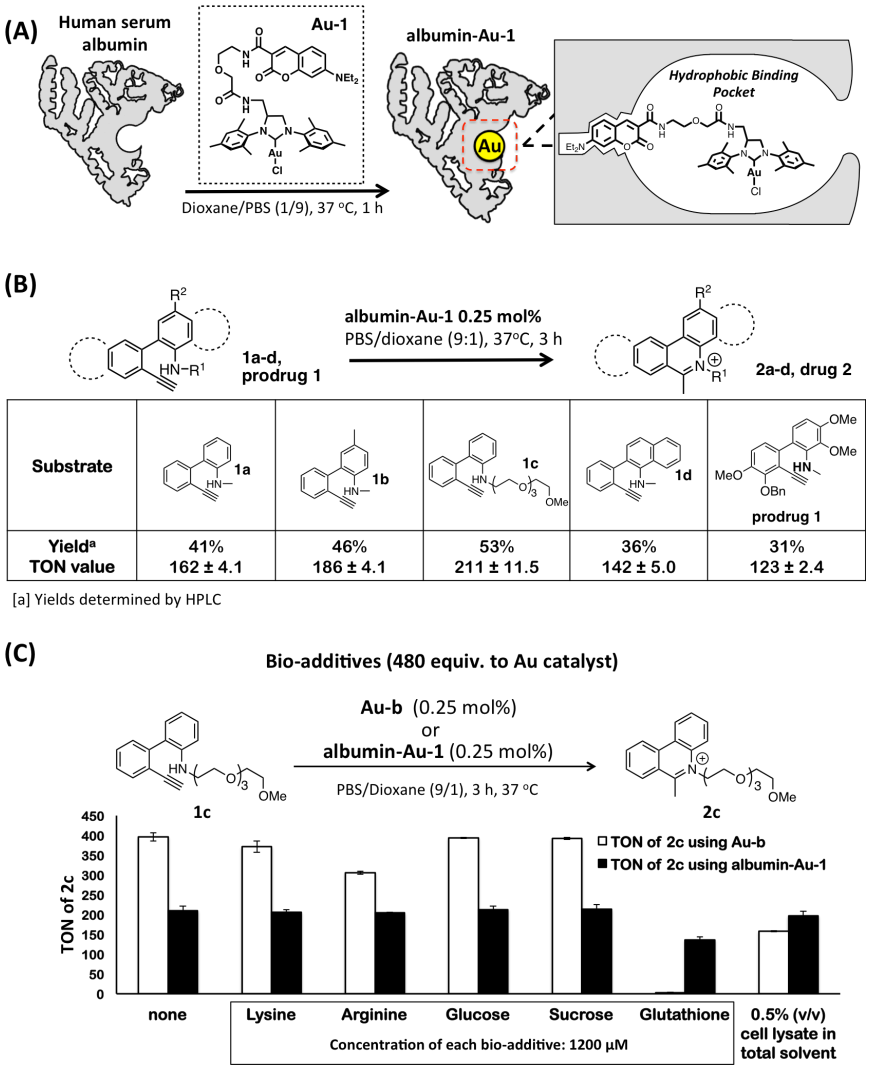

Figure 3. (A) Preparation of the albumin-based gold ArM (albumin-Au-1). (B) Hydroamination reactivity of albumin-Au-1. (C) Biocompatibility of albumin-Au-1.

As to the reactivity of **albumin–Au-1,** even though a small amount of **albumin–Au-1** (0.25 mol%) was used, it still gave good conversion yields (31–53%) and excellent TONs (over 140) with substrates **1a-d** and prodrug **1** (Fig 3B). The data prove that **albumin–Au-1** could be used as a trigger for activation of prodrug **1.** We next focused on proving biocompatibility by the one-by-one addition of several kinds of bio-additives to the gold-mediated reaction. As depicted in Fig 3C, significant changes to these measured TONs of **2c** using **albumin–Au-1** were not observed in the presence of amino acids, sugars, and 0.5% cell lysates. The TON of **2c** was still > 100 when GSH was used. By contrast, the reactivity of **Au-b** was completely quenched by the same concentration of GSH. In the presence of 0.5% cell lysate in the solvent, an approximate 50% decrease in the reactivity of **Au-b** was observed. Given the promising results *in vitro*, **albumin–Au-1** should be a more suitable trigger than **Au-1** for the prodrug strategy in the context of cancer therapy.

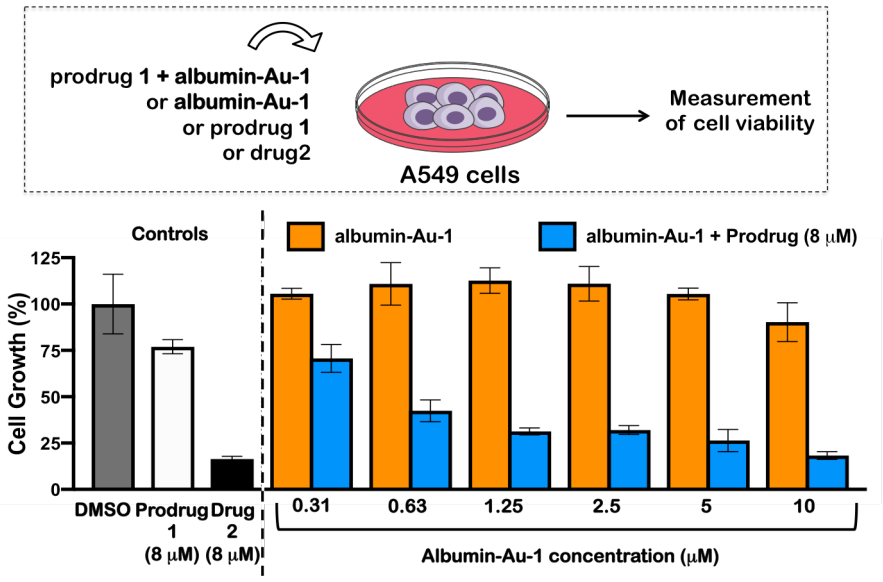

Figure 4. Cytotoxicity studies for prodrug activation via **albumin-Au-1**.

In the final stage of the study, we investigated the feasibility of using **albumin–Au-1** as a trigger to activate prodrug **1** against cancer cells. As shown in Fig 4, the mixture of **albumin–Au-1** and prodrug **1** were found to reduce A549 cell growth in a concentration-dependent manner. The data clearly showed that a gold-based ArM can be used as a trigger for the synthesis of a phenanthridinium-based drug via hydroamination, and that the resultant agent can be applied to cancer therapy, further highlighting the potential of albumin-based gold ArM to be used therapeutically.

Taken together, the work conducted in this study will significantly advance two research fields: metal-mediated prodrug strategy for cancer therapy and research focused on development of therapeutic ArMs. First, our prodrug strategy based on direct drug synthesis also represents a significant addition to the toolbox of prodrug strategy, which relies heavily on uncaging strategies. Second, current examples highlighting the therapeutic potential of ArMs have been limited[2]. The synthesized drug via the gold ArM exerted a therapeutic effect in cell-based assays, highlighting the potential usefulness of the gold ArM in anticancer applications.

The paper presenting these findings has been finalized and published (*Angew. Chem. Int. Ed.* **2021**, *60*, 12446-12454.)

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　2件／うちオープンアクセス　0件）

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２．論文標題 Enantioselective synthesis of cyclic and linear diamines by imine cycloadditions	５．発行年 2020年
３．雑誌名 Chirality	６．最初と最後の頁 1160～1168
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/chir.23265	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

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２．論文標題 Bioorthogonal release of anticancer drugs via gold-triggered 2-alkynylbenzamide cyclization	５．発行年 2020年
３．雑誌名 Chemical Science	６．最初と最後の頁 10928～10933
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D0SC04329J	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

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オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Sianturi Julinton、Manabe Yoshiyuki、Li Hao‐Sheng、Chiu Li‐Ting、Chang Tsung‐Che、Tokunaga Kento、Kabayama Kazuya、Tanemura Masahiro、Takamatsu Shinji、Miyoshi Eiji、Hung Shang‐Cheng、Fukase Koichi	４．巻 58
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３．雑誌名 Angewandte Chemie International Edition	６．最初と最後の頁 4526～4530
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/anie.201812914	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Li Pei-Jhen、Anwar Mohammad Tarigue、Fan Chen-Yo、Juang Duane S.、Lin Hsin-Yi、Chang Tsung-Che、Kawade Sachin Kisan、Chen Hsiang-Jung、Chen Yu-Ju、Tan Kui-Thong、Lin Chun-Cheng	４．巻 21
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３．雑誌名 Biomacromolecules	６．最初と最後の頁 815～824
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.biomac.9b01495	査読の有無 有
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2019年

〔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