News Release

Title

Development of **Photo-releasable "Smart ADC"** to Overcome Tumor Heterogeneity; Establishment of a new concept and technology that can eradicate cancer through the dual action of the optical bystander effect and near-infrared photoimmunotherapy

Key Points

- Antibody drugs, antibody-drug conjugates, and antibody-light absorber adducts (near-infrared photoimmunotherapy) for cancer, all of which have been under development in recent years, are antibody-based therapies that exert their selective therapeutic effects by binding to the target cancer antigen. However, due to their high targetability, there is a clinical challenge that their efficacy is limited by antigen heterogeneity, one of the tumor heterogeneities.
- We have developed a new technology (already patented) for photo-drug-releasing technology from non-cleavable linker ADCs (antibody drug conjugates), which have already been approved for clinical use, after accumulation at the cancer site by photo-irradiation.
- The drug released by light exerts its anti-tumor effect in the vicinity of the drug, and this novel mechanism of effect was named the Photo-bystander effect.
- A new concept of treatment technology has been established in which light irradiation at the site of cancer causes cancer cells expressing cancer target antigens to be crushed by light under the effect of near-infrared photoimmunotherapy, while at the same time photo-releasing an anticancer drug to induce cell death with the anticancer drug in cancer cells that are difficult to attach antibodies (low or no cancer antigen expression). This therapeutic concept is expected to overcome treatment resistance due to heterogeneous antigen expression, which is one of the tumor heterogeneities.

Summary 1

Former Graduate student **Kazuomi Takahashi (co-first author 1)** in the department of respiratory medicine (professor ***Ishii Makoto***) (now Chubu Rosai Hospital), Designated Lecturer **Kazuhide Sato (co-first author, corresponding author/ last author)** Nagoya University Graduate School of Medicine, in the department of respiratory medicine, Institute for Advanced Research, have succeeded in developing a photoreleasable Smart ADC that, after accumulating at the cancer site, releases the drug into the

surrounding area while destroying the cancer with NIR-light. This photoreleasable Smart ADC has the potential to be a new technology to overcome treatment resistance caused by tumor heterogeneity.

Solid tumors are known to be heterogeneous, with cancer cells having diverse properties, and overcoming this heterogeneity has been a challenge as a cause of treatment resistance and recurrence. Antibody therapy against cancer has had limited effectiveness due to this heterogeneity of cancer, which results in the heterogeneous expression of antigens on the surface of targeted cancer cells. Recently, antibody drug conjugates (ADCs), which are armed antibodies, have attracted attention as new therapeutic agents. Drugs include anticancer agents and optical absorbers, which have been developed in a variety of ways. However, there has been a clinical problem that the use of tumor-targeting antibodies has limited efficacy due to the heterogeneity described above.

Near-infrared photoimmunotherapy is a new cancer treatment established in 2011 by Dr. Hisataka Kobayashi et al. of the National Institutes of Health/National Cancer Institute (NCI/NIH) in the United States, who are also co-researchers. A complex of an antibody that specifically recognizes proteins expressed by cancer cells and IR700, a light-absorbing substance, is synthesized, and when irradiated with near-infrared light at around 690 nm while bound to the target protein on the cell surface, the cancer cells are destroyed. As a fifth cancer treatment, it is a promising new therapeutic technology, and in September 2020, it was the first in the world to be approved in Japan for recurrent and previously treated head and neck cancer with high expression of EGFR, and was included in the insurance coverage. Since this therapy also uses antibodies, it falls under the category of ADC mentioned earlier and has the common problem of tumor heterogeneity.

In this study, we succeeded in releasing a derivative of DM1, a bound non-cleaved anticancer drug, by photoirradiation using a simple method of further adding IR700 to T-DM1 (Kadcyla), an ADC with an anticancer drug attached to the antibody by a non-cleaved thiol linker, which is already approved and used in clinical practice. In addition, since IR700 shatters cancer cells bound by the effect of near-infrared photoimmunotherapy through NIR-light irradiation, it can induce cell death by the anticancer action of DM1 derivatives released from surrounding cancer cells to which antibodies are bound and unbound by light, which may lead to radical cure of solid tumors in which cancer target antigen is heterogeneously expressed.

This research was supported by the Program for Developing Next-generation Researchers (Japan Science and Technology Agency); JSPS KAKENHI (18K15923, 21K07217); CREST; FOREST-Souhatsu (JST); The Uehara Memorial Foundation; Noguchi Shitagau Award; Takeda Science Foundation; and Princess Takamatsu Cancer Research Fund, Yakurikigaku Foundation, etc.

The paper was published in *Bioengineering & Translational Medicine*, which is an official journal of the

American Institute of Chemical Engineers (AIChE) and its technological community, the Society for Biological Engineering (SBE) (electronic version dated Aug. 22, 2022).

Summary 2

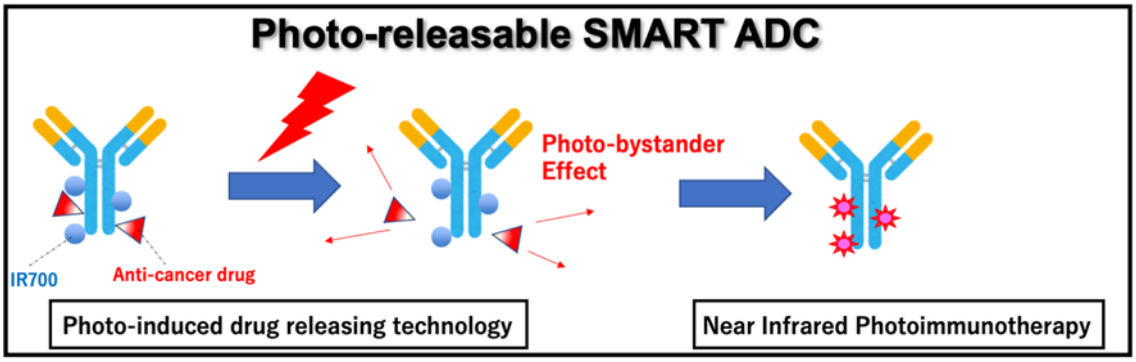
Research Background

In recent years, antibody-drug conjugates, in which an antibody is combined with a drug, photo-absorber, or nuclide, have become widely used in the treatment of cancer, and are attracting attention as a new field of anticancer drugs. Antibody Drug Conjugates (ADC), in which an antibody is linked to an anticancer drug by a linker, have been developed and implemented by pharmaceutical companies in Japan. The use of this technology has been attracting attention in clinical oncology.

NIR-PIT is a new cancer treatment method developed by Dr. Hisataka Kobayashi and his colleagues at the National Institutes of Health/National Cancer Institute (NCI/NIH) in 2011. A complex of antibodies that specifically recognize proteins expressed by cancer cells and a photosensitive substance IR700 is synthesized, and irradiated with near-infrared light around 690nm while bound to the target proteins on the cell surface, the cells are destroyed. These cell death mechanisms have been clarified in 2018 by Sato et al, the lead author of this release, as a new concept of cell death based on photochemical reactions (Sato K, et.al. ACS Cent Sci. 2018 Nov 28;4(11):1559-1569. doi: 10.1021/acscentsci.8b00565.). Since it can target and destroy cancer cells in a different way, it is expected to be the "fifth cancer treatment" following surgery, radiation, chemotherapy, and cancer immunotherapy. NIR-PIT was first approved in Japan in September 2020 for the treatment of recurrent and previously untreated head and neck cancer that highly expresses EGFR under the PMDA's Pioneer Application System and Early Approval System. Near-infrared phototherapy also utilizes a photo-absorber to an antibody, and in a broad sense, this therapy can also be considered an ADC-based therapy.

However, antibody therapy against target antigens on the surface of solid tumors poses a clinical challenge in that its effectiveness is limited by the heterogeneous expression of target antigens, which is one of the heterogeneities of solid tumors due to their highly targeted nature. Various studies have been conducted to overcome tumor heterogeneity, but they have not been successful, leading to cancer recurrence and limited therapeutic efficacy.

In this study, we succeeded in creating a new concept to overcome the heterogeneity of target antigen expression in cancer described above, by successfully using a simple method to photo-release only the drug at the cancer site by ADC and simultaneously demonstrating the effect of near-infrared photoimmunotherapy. We named this ADC "Photo-releasable Smart ADC". We also named its special effect as Photo-bystander effect.



Research Results

We conjugated Trastuzumab (Tra) which is HER2 targeting antibodies, to IR700, and Trastuzumab-emtansin (T-DM1) which is an ADC of Tra, to IR700. T-DM1 is a stable, non-cleavable, thiol-binding SMCC that structurally releases DM1 in vivo only after uptake into the bound cancer cells. The antibody and DM1 are known to be bound by a linker. After intravenous injection, both remain stable at the cancer site, bind to HER2 on the cancer cell surface, and enter the cancer cells, both of which were previously reported (Sato K, et. al. ACS Cent Sci. 2018 Nov 28;4(11):1559-1569. doi: 10.1021/ (acscentsci.8b00565.) When T-DM1-IR700 was irradiated with near-infrared light and mass spectrometry was performed, nothing was detected without irradiation, but with 16 (J/cm2) light irradiation, a derivative of DM1 was detected from T-DM1-IR700 by light irradiation. This mechanism was found to be applicable to antibodies against specific targets of various cancers, since it is released even with different antibodies.

Next, to model tumor heterogeneity, we constructed a mixed tumor model of 3T3/HER2 cells that express high levels of HER2 and MDAMB468 cells that do not express HER2. In vitro and in a mouse tumor model, the combination of T-DM1-IR700 and near-infrared light exerted the cytotoxic effect on the unbound MDAMB468 cells or tumors. This effect was named the Photo-bystander effect. In animal models, survival was prolonged and some individuals were cured completely.

The above results show that near-infrared photoimmunotherapy can break down target cancer cells with antibodies, and at the same time, anti-cancer drugs released by NIR-light irradiation can cause cancer cells that have leaked from the target to die by the photo-bystander effect, which is a new approach to overcome treatment resistance caused by the heterogeneous expression of cancer.

Research Summary and Future Perspective

In this study, we succeeded in creating a new concept to overcome the heterogeneity of target antigen

expression in cancer with using a simple method. This technology causes photo-drug-release at the cancer site from ADC and simultaneously demonstrating the effect of near-infrared photoimmunotherapy. We expect that this new novel technology to overcome cancer heterogeneity will lead to the eradication of cancer. In addition, since the ADC used in this research has already been approved for cancer treatment and NIR-PIT has also received limited approval, this treatment method is expected to have easy clinical application. In addition, by changing the antibodies, the new method can be applied to all types of cancers, and thus is expected to contribute to a wide range of cancer therapies.

This therapeutic concept is expected to be used in the future in clinical practice as a step forward in the antibody therapy for solid tumors, where recurrence is a problem due to heterogeneous expression of cancer-specific target antigens, and as a new method to enhance the efficacy of near-infrared photoimmunotherapy, which has just been implemented.

Publication

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Near-infrared-induced drug release from antibody–drug double conjugates exerts a cytotoxic photo-bystander effect

Bioengineering & Translational Medicine

DOI; 10.1002/BTM2.10388

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Bio_220822.pdf