News Press Research Release

Title

Spatiotemporal depletion of tumor-associated immune checkpoint PD-L1 with near-infrared photoimmunotherapy promotes antitumor immunity

Key Points

- We successfully evaluated and developed near-infrared photoimmunotherapy targeting PD-L1, an immune checkpoint molecule, and found that PD-L1-targeted near-infrared photoimmunotherapy synergistically exerts significant anti-tumor effects via cancer immunity, despite the limited expression of PD-L1. We also found that metastatic tumors without light irradiation also exhibited antitumor effects via cancer immunity. Translated with www.DeepL.com/Translator (free version)
- We have shown that near-infrared photoimmunotherapy targeting PD-L1 synergistically activates anti-tumor immunity by light-induced destruction of cancer cells, activation of tumor immunity by immune checkpoint molecules, and modification of the cancer microenvironment.
- This study proposes near-infrared photoimmunotherapy as an alternative treatment for patients who are not eligible for near-infrared photoimmunotherapy based on high expression of cancer-specific antigens, and is expected to contribute as a basic knowledge for the application of near-infrared photoimmunotherapy targeting PD-L1 to humans.

Summary 1

Graduate student Shunichi Taki (1st author) at the department of Respiratory Medicine, Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu),, Assistant Professor Kazuhide Sato (corresponding author), at Institute for Advanced Research, Nagoya University (Director: Yoshiyuki Suto), and their collaborators succeeded in developing near-infrared photoimmunotherapy(NIR-PIT) targeting PD-L1, an immune checkpoint molecule.

PD-L1, a ligand for PD-1, an immune checkpoint protein, has been detected in various solid tumors, and immune checkpoint inhibitors targeting PD-1/PD-L1 have shown efficacy in cancers of various organs. Even if the expression of PD-L1 in tumors is low (TPS (Tumor Proportion Score) of ≥1%), they can be used as first-line therapy and are known to have certain efficacy. However, the effect is not sufficient, and there is a need to develop technology that can enhance the effect of immune checkpoint inhibitors.

NIR-PIT is a new cancer treatment reported in 2011 by Dr Hisataka Kobayashi and his colleagues at the National Cancer Institute (NCI/NIH). 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 cancer cells are destroyed. It is a novel therapeutic technology that is expected to become the fifth cancer treatment, and was approved in Japan in September 2020 for the treatment of recurrent and previously treated head and neck cancer that highly expresses EGFR, ahead of the rest of the world. For the future expansion of indications, there is a need to consider new targets, and especially the combination with cancer immunotherapy is considered to be ideal.

In this study, we synthesized a complex of mouse anti-PD-L1-F(ab')$_2$ antibody and IR700, and demonstrated the effects of NIR-PIT on tumors by targeting PD-L1 in cell and animal experiments. Although PD-L1 expression is low in mouse tumors as well as in humans, sufficient anti-tumor effects were obtained by activating cancer immunity. It was also found that the treatment had a certain effect on tumors in metastatic areas (photo-abscopal effect). This treatment is expected to be used in clinical practice in the future as an application of NIR-PIT to patients whose tumors do not have high expression of specific target antigens.

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Summary 2

Research Background

PD-L1, a ligand for the immune checkpoint protein PD-1, is detected in a variety of solid tumors. PD-L1 is present in tumor cell membranes and works to weaken the immune response of CD8(+) T cells and evade immune surveillance. PD-1/PD-L1-targeted immune checkpoint inhibitors have been shown to be effective in the treatment of cancers of various organs, and have some efficacy as first-line therapy even when the tumor expression of PD-L1 is low (TPS (Tumor Proportion Score) ≥1%). However, the effect is not sufficient, and there is a need to develop technology that can enhance the effect of immune checkpoint inhibitors.

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 prior application system.

However, conventional NIR-PIT, including this approval, requires cancer-targeting antigens that are highly expressed on cancer cells, which limits the number of patients who can receive the therapy. It is necessary to deliver this innovative light-targeted cancer therapy to a wide range of patients so that it can be used as a general standard treatment. To solve this problem, we focused on PD-L1, an immune checkpoint molecule that is not highly expressed but is widely expressed in low to moderate levels in cancer, and planned to develop NIR-PIT targeting cancer immunity. In addition, we found that this treatment method also enhances the effect of cancer immune checkpoint inhibitors (Figure 1).

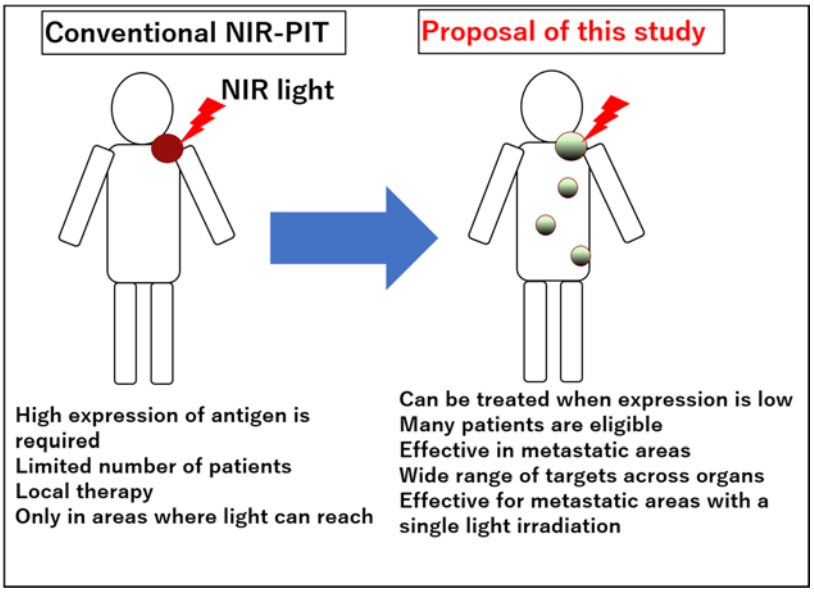

Figure 1. Aim of this research

Research Results

We prepared PD-L1 F(ab')$_2$-IR700 by synthesizing a complex of PD-L1 F(ab')$_2$ and photosensitizer IR700. PD-L1 F(ab')$_2$-IR700 is thought to reduce systemic side effects by eliminating non-specific binding of PD-L1 antibodies. It is also smaller in size than IgG and is expected to have higher tumor penetration. PD-L1

$F(ab')_2$-IR700 was used to treat murine tumor cells (lung cancer, colorectal cancer, prostate cancer, melanoma). The expression of PD-L1 was low (about 1/100) compared to the expression of EGFR in tumors, which is currently approved in humans. Since the effect of this therapy depends on the expression level of cancer antigens on the surface of the cancer cell membrane, the effect of NIR-PIT targeting PD-L1 in cell experiments was limited compared to that in tumors with high expression of EGFR, and more intense light energy was required. The effect of NIR-PIT was also observed in cell experiments when sufficient light energy was provided. In summary, the effects of NIR-PIT targeting PD-L1 in cell experiments were limited and not suitable for therapeutic application.

However, when we examined the effects of single NIR-PIT using PD-L1 $F(ab')_2$-IR700 in a mouse syngeneic tumor model, we found significant suppression of tumor growth and prolongation of survival, which was not expected from the effects of cell experiments. Furthermore, in a mouse syngeneic metastasis model, irradiation of only one tumor with near-infrared light resulted in suppression of tumor growth not only in the irradiated tumor but also in the tumor not irradiated with near-infrared light, and significantly prolonged survival.

We continued to analyze anti-tumor immunity to elucidate the discrepancy between the therapeutic effects of cell and animal experiments described above. It was confirmed that CD8(+) T cells and NK cells, which are key players of anti-tumor immunity, were activated inside the tumor. In addition, detailed analysis of the tumor microenvironment revealed that bone marrow-derived immunosuppressive cells (MDSCs) were reduced. We found that the partial tumor necrosis induced by NIR-PIT, the immune checkpoint effect of PD-L1 $F(ab')_2$, and the removal of MDSCs from the tumor microenvironment acted synergistically to activate anti-tumor immunity and exert a remarkable anti-tumor effect. In addition, analysis of the blood of mice suggested that the anti-tumor immunity was enhanced systemically. This systemic activation of anti-tumor immunity was thought to have had an effect on tumors that had not been treated with NIR-PIT and that had not been exposed to light (Figure 2).

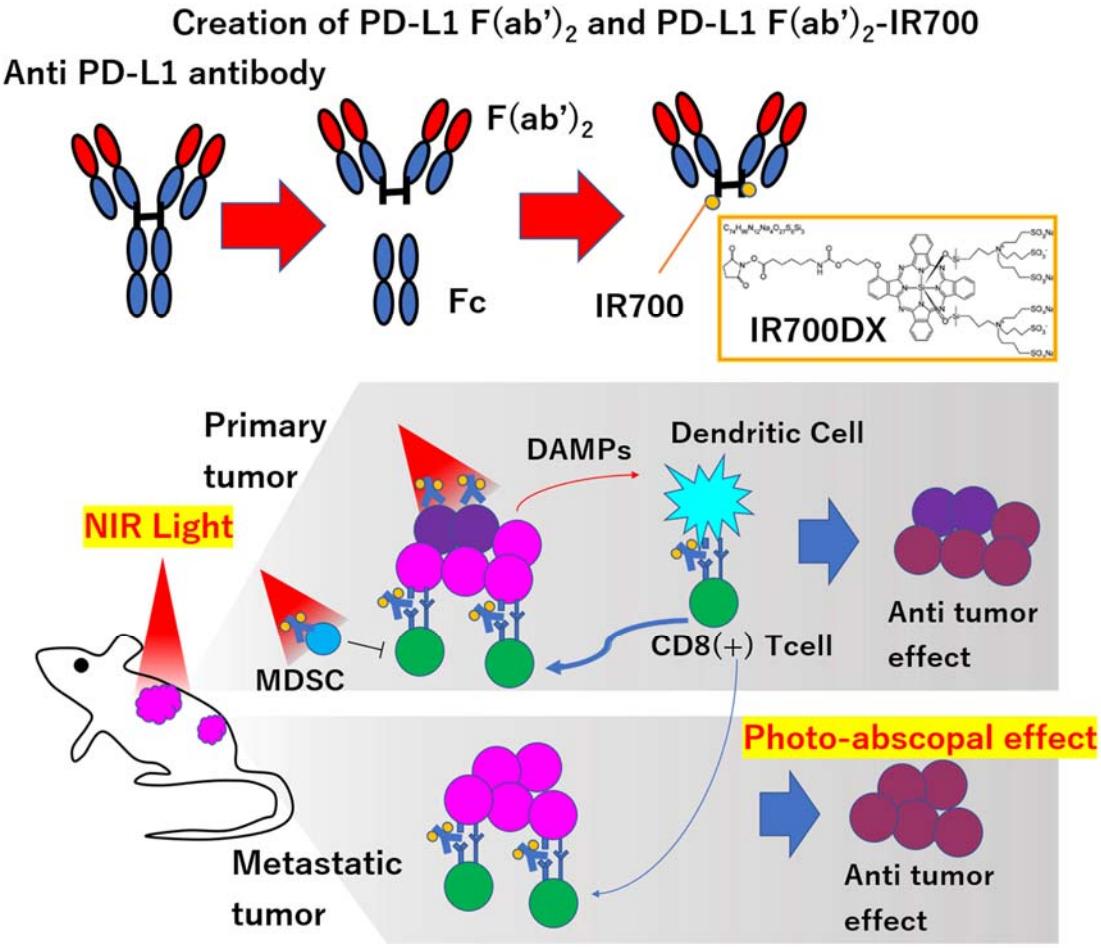

Figure 2. Creation of PD-L1 F(ab')$_2$ and development of NIR-PIT application to tumors and its mechanism.

Research Summary and Future Perspective

NIR-PIT targeting PD-L1 showed sufficient anti-tumor effects even at low to moderate expression levels. Furthermore, NIR-PIT targeting PD-L1 was shown to have anti-tumor effects not only at the site of exposure to near-infrared light but also on metastatic tumors. The results of this study indicate that NIR-PIT can be applied to patients without high expression of appropriate specific cancer antigens, as it is considered to be a next-generation type of cancer immunotherapy that differs in concept from conventional NIR-PIT targeting highly expressed targets. Thus, it is considered to be possible to expand the indication to a wide range of cancer patients, and since PD-L1 antibody is already clinically approved, this treatment method is considered to be easy to apply clinically.

This treatment is expected to be used in the future in clinical practice as a proposal of NIR-PIT as an alternative treatment for patients who are not eligible for NIR-PIT based on the application of high

expression of cancer-specific antigens. This study will contribute to the basic knowledge for the implementation of NIR-PIT targeting PD-L1 to human malignant tumors.

Publication

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