News Release

Title

Development of Image-based Biomarker for Treatment Confirmation of Near-Infrared Photoimmunotherapy Using Microbubble Contrast-Assisted Ultrasound Images ~ New Method and New Concept for Easily Confirming Appropriate NIR-light irradiation ~

Key Points

- In Near-infrared Photoimmunotherapy (NIR-PIT), which is considered the fifth cancer therapy, there is a need for a biomarker that can confirm the appropriate NIR-light irradiation and predict therapeutic efficacy.
- There is a need for a biomarker that can predict treatment efficacy on the spot where the procedure is performed, using a simple method.
- As a novel concept, we found that tumors after NIR-PIT have a [micro-size super-EPR effect], in which micro-size particles are retained in the tumor, transcending the conventional EPR effect (Enhanced Permeation and Retention Effect). Previously, solid tumors were known to have an EPR effect, but the size of the EPR effect is considered to be about 20 - 200 nm.
- By linking this novel concept to a new technology that uses microbubbles to be evaluated by contrast enhanced ultrasound imaging, it was found that in NIR-PIT, the degree of therapeutic effect can be estimated after light irradiation.
- This image biomarker can be evaluated by combining a microbubble contrast agent, which has already been approved as a diagnostic contrast agent for ultrasound examination of liver tumors, with an ultrasound equipment, which is expected to reduce the hurdles to clinical application and increase the potential for implementation.
- This new imaging biomarker is expected to improve the appropriate efficacy of NIR-PIT.

Summary 1

Designated Lecturer **Kazuhide Sato (corresponding author/ last author)** Nagoya University Graduate School of Medicine, Institute for Advanced Research, and Former Graduate student Kohei Matsuka **(first author)** in the division of host defense sciences, department of Integrated Health Sciences (professor ***Mitsuo Sato***) have succeeded in developing image-based biomarker for treatment confirmation of Near-Infrared Photoimmunotherapy (NIR-PIT) using microbubble contrast-enhanced ultrasound images.

NIR-PIT 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.

In NIR-PIT, there is a need to irradiate NIR-light, and light irradiation is performed by setting a sufficient amount of light intensity. However, uniform irradiation is difficult because light is attenuated by reflection and scattering in the tissue, and an indicator that can appropriately determine the completion of light irradiation has been sought. Therefore, this research group focused on the fact that the EPR effect is enhanced in tumors treated with NIR-PIT, and newly discovered that the expansion of the para-vascular space after treatment allows the retention of even micro-sized particles, and clarified the mechanism and the upper limit of the particle size to be retained.

Using this new concept, we also thought that by applying ultrasound imaging equipment and its microbubble contrast agent, this technology could be used as an imaging bio-marker to confirm and predict treatment effects after NIR-light irradiation, and if insufficient, additional irradiation could be performed

flexibly, for example. Since an ultrasound imaging equipment has already been introduced in most hospitals and the microbubble contrast agent used in this study has already been approved for a diagnostic contrast agent, we think that this technology is easy to translate into the clinic.

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The paper was published in ***Ebiomedicine (Cell Press, and the Lancet),*** which is a journal of medical science (electronic version dated on August 7).

Summary 2

Research Background

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.

As described above, NIR-PIT is expected to be a new cancer treatment modality, but if NIR-light irradiation is insufficient, the therapeutic effect may be insufficient and additional treatment may be required or the cancer

may recur. Uniform irradiation is difficult because light is attenuated by reflection and scattering in the tissue, and an indicator that can appropriately judge the completion of light irradiation has been sought. In addition, if it is possible to determine whether light irradiation is being performed properly during the irradiation procedure, additional light irradiation can be performed on the spot as needed, which is considered to be highly beneficial to the patient. Therefore, there was a need to develop a technology that can determine whether or not light irradiation is appropriate during or immediately after the light irradiation procedure.

This study provides a discovery that SUPR enhances the intra-tumour persistence of microparticles
with a diameter of up to 5 mm, approximately, which we named as the micro-sized SUPR (super enhanced permeability and retention) effect. Moreover, an image-based marker for monitoring and confirming the effectiveness of NIR-PIT using micro-bubble and contrast-enhanced ultrasound (CEUS) could be realised and the method could be easily adapted for clinical use (Figure A).

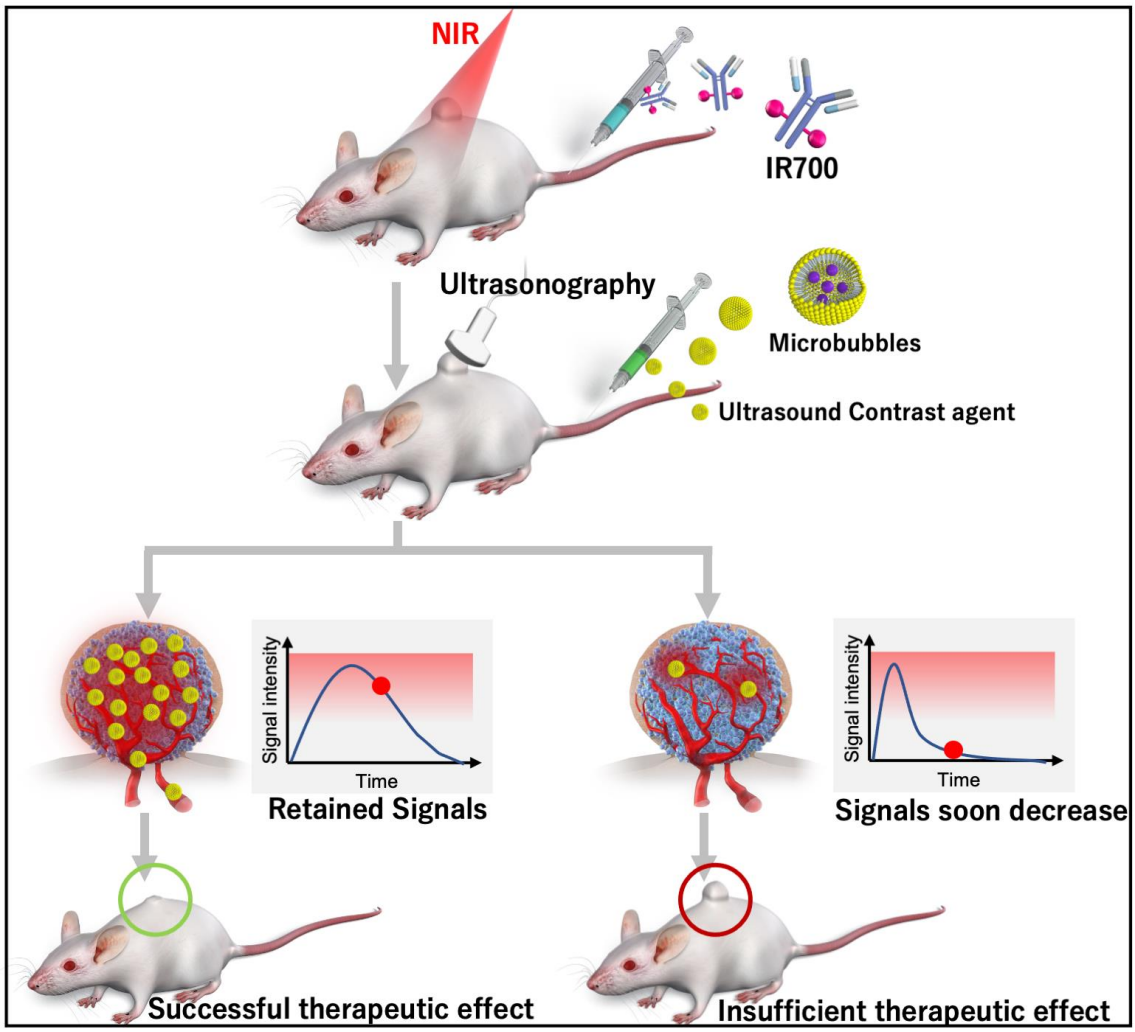

Figure A. Prediction of therapeutic effect of NIR-PIT using microbubble contrast-enhanced ultrasound (CEUS) images

Research Results

Tumors treated with NIR-PIT have an increased EPR effect, which is named the SUPR (super enhanced permeability and retention) effect. Conventionally, the EPR effect in tumors is limited to nano-sized particles, and particles with sizes of approximately 20 nm to 200 nm are known to stay in tumors. Anticancer drugs based on this EPR effect have already been used in the clinic.

First, in this study, we confirmed that fluorescent nanoparticles, 800 nm quantum dots (20-30 nm), accumulate in tumors treated with NIR-PIT by SUPR. When the treated tumors were made transparent using the CUBIC

method, a transparency technique, the enlargement of the vascular area was proven by 3D image analysis. Based on this, we hypothesized that particles of larger sizes could be retained, and investigated with fluorescent particles (NIR-PLGA) of 2 mm and 5 mm sizes, and newly found that retention increased with both sizes. We also found that the higher the retention, the higher the anti-tumor effect of NIR-PIT. The micro-sized SUPR effect concept (micro-sized SUPR effect) is novel, and we have applied this mechanism to a new technology to evaluate the retention of microbubbles, which are 2 mm in size, using ultrasound imaging. We found that the greater the microbubble retention, the greater the therapeutic effect of NIR-PIT, and this simple method can estimate the degree of therapeutic effect immediately after NIR-light irradiation (Figure A).

Research Summary and Future Perspective

Contrast-enhanced ultrasonography (CEUS), an ultrasonography using microbubble contrast agent, is a medical technique widely used in hospitals as a diagnostic agent and method for liver tumors. The hurdle for this method to confirm the therapeutic effect of NIR-PIT by the retention of the microbubble contrast agent developed this time is considered to be low from both soft and hard aspects. Therefore, further optimization of this evaluation technique, as well as basic studies and non-clinical studies to move toward clinical trials, is expected to lead to more appropriate treatment of NIR-PIT and an increase in its effectiveness. We will continue to develop the technology as a biomarker for NIR-PIT originating in Japan, and hope to contribute to patients.

Publication

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Contrast-enhanced ultrasound imaging for monitoring the efficacy of near-infrared photoimmunotherapy

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https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Ebi_230807.pdf