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Press Release

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National Institutes of Biomedical Innovation, Health and Nutrition

Fields: Life Sciences / Pharmaceutical Sciences

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Production of "Epitope region-bridging biparatopic antibody"

-Improving the functionality of TNFR2 antagonist through 1:1 binding design-

A group of scientists in National Institutes of Biomedical Innovation, Health and Nutrition (hereinafter referred to as "NIBIOHN") , Kyoto University, Osaka University, and the University of Tokyo has successfully created a biparatopic antibody (BpAb) that exhibits outstanding functionality as a receptor function inhibitor (antagonist) for tumor necrosis factor receptor type 2 (TNFR2).

BpAb is an artificial bispecific antibody that has been engineered to bind to two different antibody-binding regions (epitope regions) on a single target molecule that cannot be accessed simultaneously by natural antibodies. Natural antibodies with the ligand blocking activity (antagonist function) inevitably shows the weak level of undesired receptor-stimulating activity (agonist function). The undesired effects were concerned for the potential side effects. In this research, the research team led by Dr. Hiroki Akiba, Dr. Satoshi Nagata, and Dr. Kouhei Tsumoto successfully produced a BpAb that binds to TNFR2 in a 1:1-manner, eliminating the agonist function, thereby functions as an excellent antagonist. The unique binding mode was confirmed using cryo-electron microscopy. TNFR2 is known to be involved in suppressing tumor immunity through activation of regulatory T cells. Therefore, the antagonist BpAb is expected to be developed as a therapeutic drug for cancer treatment.

This technology is expected to be applied widely to other receptors and to promote the development of highly functional antibody drugs.

The results of this research were published online in *Communications Biology* on September 27, 2023.

Website: https://www.nature.com/articles/s42003-023-05326-8

❖ Background and significance of the research

The type 2 TNF receptor (TNFR2) has primarily expressed in regulatory T cells and plays an important role in their proliferation. Regulatory T cells, when activated within cancer tissues, inhibit the function of the immune system and lead to the progression of cancer. Therefore, TNFR2 antagonists show promise as anticancer drugs for various cancer types.

TNFR2 is activated through forming clusters on the cell membrane. In natural systems, three TNFR2 bind to the natural trimeric ligand TNFα to oligomerize, and a larger cluster is formed. When antagonistic antibodies are developed, this mechanism itself may be an obstacle. Since natural antibodies also have two

antigen-binding regions, both binding regions simultaneously bind to TNFR2 to promote its activation (Figure 1).

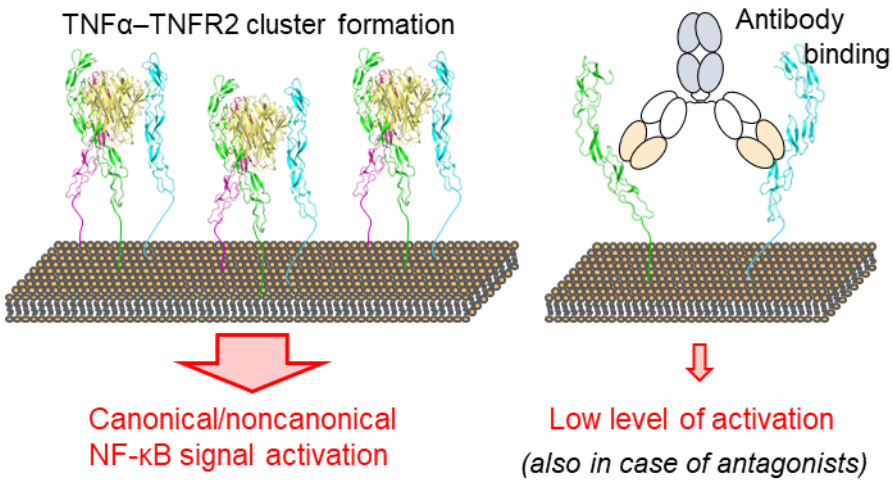

Figure 1. Activation of TNFR2 by TNFα (left) and weak activation (side reaction) of TNFR2 that is inevitable with natural antagonist antibodies (right)

To overcome this obstacle, the group of scientists developed a biparatopic antibody (BpAb). BpAb is an artificially designed molecule that binds to two different epitopes on a target antigen molecule. BpAbs may form crosslinks between multiple antigen molecules (left) or bind antigen in a 1:1-manner (Figure 2). BpAb with 1:1-binding property may function an antagonist without TNFR2 activation. In this study, they successfully developed a 1:1-binding antagonist, Bp109-92, from comprehensively produced BpAbs from a panel of five antibodies binding different epitope regions. Bp109-92 was capable of inhibiting the TNFα-dependent proliferation of regulatory T cells. Binding mode of this BpAb was also analyzed in single-particle cryo-electron microscopy (Figure 3).

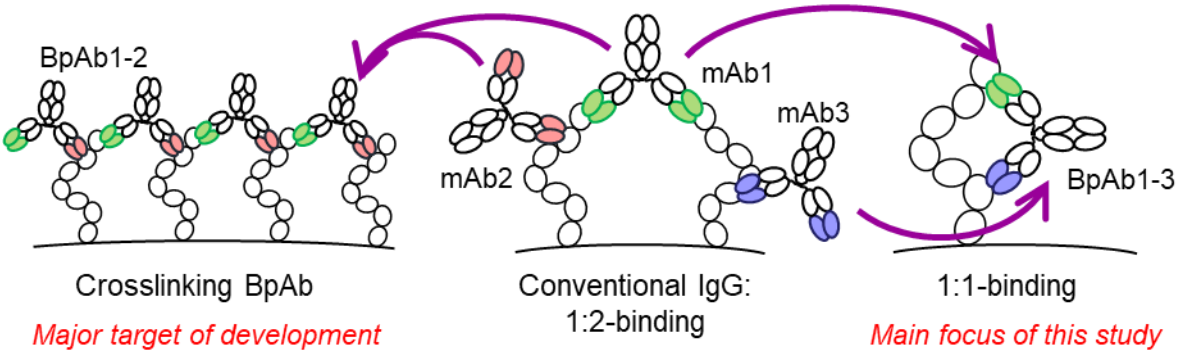

Figure 2. Antigen binding mode of biparatopic antibodies (BpAbs). Natural antibodies form a 1:2 antibody:antigen complex, whereas BpAb can form a cross-linked complex or a 1:1 complex. Excellent function of the latter was demonstrated in this study.

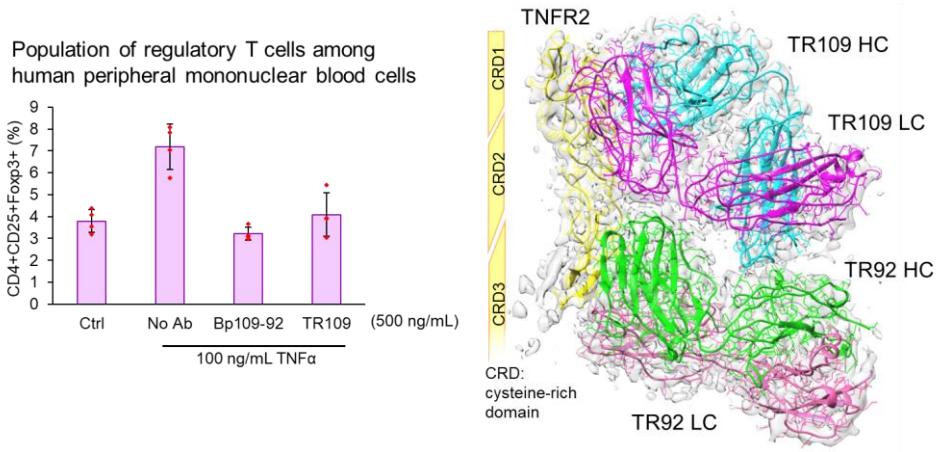

Figure 3. Antagonistic function of the 1:1-binding BpAb, Bp109-92, against TNFα-dependent proliferation of regulatory T cells (left) and the structure of Bp109-92–TNFR2 complex (right).

❖ Future prospective

The TNFR2 antagonist designed and produced in this study can be developed as an anticancer agent, and is now under investigation. Development of BpAbs against other target antigen molecules with a similar design is also expected to expand their application to therapeutics.

❖ Research support

This study was supported partially by Japan Agency for Medical Research and Development (AMED) grant numbers JP22ak0101099, JP21am0101117, JP22ama121003, JP17pc0101020, Japan Science and Technology Agency (JST) grant number JPMJOP1861, Japan Society for the Promotion of Science (JSPS) grant numbers JP21K06453, JP20K22630, Kyoto University Foundation, Takeda Science Foundation, and JEOL YOKOGUSHI Research Alliance Laboratories of Osaka University.

❖ Article information

Title: Development of a 1:1-binding biparatopic anti-TNFR2 antagonist by reducing signaling activity through epitope selection

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❖ Conflict of interest

Hiroki Akiba, Satoshi Nagata and Kouhei Tsumoto filed a patent related to the described biparatopic antibodies (PCT/JP2021/013341). Haruhiko Kamada and Satoshi Nagata are co-founders of Epitope Science Co., Ltd.

❖ Related patents

#1

" EPITOPE NORMALIZED ANTIBODY PANEL, METHOD FOR CREATING SAME AND UTILIZATION THEREOF"

Proprietors : NATIONAL INSTITUTES OF BIOMEDICAL INNOVATION, HEALTH AND NUTRITION

Inventors : Hirosato KONDO、Haruhiko KAMADA、Satoshi NAGATA、Kenji MIZUGUCHI、Yoichi MURAKAMI、Kouhei TSUMOTO、Hiroki AKIBA

Patent number : JP 7054209

#2

" EPITOPE REGION-BRIDGING BIPARATOPIC ANTIBODY AND METHOD FOR PRODUCING SAME "

Proprietors : NATIONAL INSTITUTES OF BIOMEDICAL INNOVATION, HEALTH AND NUTRITION

Inventors : Hiroki AKIBA、Kouhei TSUMOTO、Satoshi NAGATA

Patent number : JP 7101433

Application number : US 17/915,837 , EP 21781490.4 , CN No 202180026766.5

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