Prevention of radionuclide-induced antibody denaturation maintains active targeting and maximizes antitumor efficacy in ^{211}At-radioimmunotherapy†

H. Takashima,[*1,*2] K. Ohnuki,[*3] S. Manabe,[*2,*4,*5,*6] Y. Koga,[*1,*7] R. Tsumura,[*1] T. Anzai,[*1] Y. Wang,[*2] X. Yin,[*2] N. Sato,[*2] Y. Shigekawa,[*2] A. Nambu,[*2] S. Usuda,[*2] H. Haba,[*2] H. Fujii,[*3] and M. Yasunaga[*1]

Selective tumor accumulation of alpha emitters with high linear energy transfer and a short particle range in tissue results in potent antitumor efficacy without serious toxicity. Thus, there is a growing interest in developing novel target alpha therapies. Astatine-211 (^{211}At) is a promising alpha emitter that is applicable to cancer treatment.

In the preparation process of radioactive antibodies, caution should be exercised in the radionuclide-induced chemical reaction causing antibody denaturation. We demonstrated that reactive oxygen species (ROS) generated from ^{211}At-induced radiolysis of water denature astatinated antibodies.[1)] The radionuclide-induced antibody denaturation disrupts binding activity and attenuates *in vivo* antitumor effect. In contrast, sodium ascorbate (SA), a free radical scavenger, successfully quenches ROS and prevents denaturation, resulting in the maintenance of binding activity and antitumor effect.[2)] Although we revealed the influence of radiochemical reaction on ^{211}At-labeled antibody as described above, several questions remain. First, it is unclear whether ^{211}At-induced denaturation affects the pharmacokinetics of radioactive antibodies, such as half-life in blood circulation, distribution to normal organs, and tumor accumulation via active targeting and passive targeting, which are based on antigen-antibody reaction and enhanced permeability and retention effect, respectively.[3)] In addition, the protective effects of SA on the pharmacokinetics have not been clarified.

In this study, using an ^{211}At-labeled anti-human epidermal growth factor receptor 2 (HER2) antibody stabilized with SA, a denatured radioactive anti-HER2 antibody, and an ^{211}At-labeled nontargeted control antibody (anti-CD20 antibody) stabilized with SA, we compared their residence time in blood circulation, distribution to normal organs, tumor accumulation, and antitumor effect in a xenograft model with high expression of HER2.

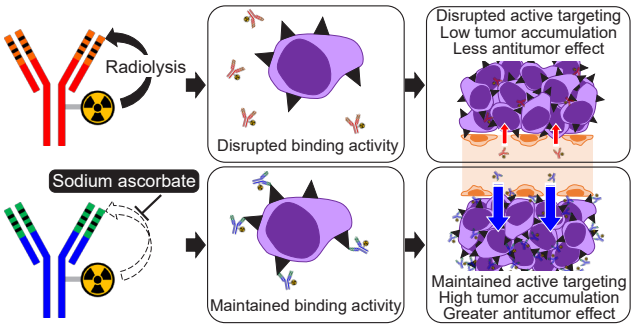

Fig. 1. Graphical abstract of this study.

In sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, we confirmed that ^{211}At denatured radioactive anti-HER2 and anti-CD20 antibodies under no protection, and SA successfully stabilized the radioactive antibodies. The binding activity of the denatured radioactive anti-HER2 antibody was consistently disrupted, whereas the binding activity of the stabilized immunoconjugate was comparable to the naked antibody. Similarly, the cytocidal effect of the denatured radioactive anti-HER2 antibody on HER2-positive cancer cells was attenuated more than the stabilized radioactive antibody.

There is no difference in blood circulation time as well as distribution to normal organs between the groups administered ^{211}At-labeled anti-HER2 antibody under SA protection and the denatured radioactive anti-HER2 antibody in *ex vivo* biodistribution study. These findings suggest that ^{211}At-induced antibody denaturation may not affect tumor accumulation via passive targeting. However, single-photon emission computed tomography and *ex vivo* biodistribution studies demonstrated that tumor accumulation of ^{211}At-labeled anti-HER2 antibody stabilized with SA was significantly higher than that of the denatured radioactive anti-HER2 antibody and ^{211}At-labeled nontargeted control antibody under SA protection. In a xenograft model with high expression of HER2, the stabilized radioactive anti-HER2 antibody consistently outperformed the denature immunoconjugate and the radioactive nontargeted control antibody. ^{211}At-induced antibody denaturation hampers

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*1 Developmental Therapeutics, Exploratory Oncology Research & Clinical Trial Center (EPOC), National Cancer Center
*2 RIKEN Nishina Center
*3 Division of Functional Imaging, EPOC, National Cancer Center
*4 Laboratory of Functional Molecule Chemistry, Pharmaceutical Department and Institute of Medicinal Chemistry, Hoshi University
*5 Research Center for Pharmaceutical Development, Tohoku University
*6 Glycometabolic Biochemistry Laboratory, RIKEN
*7 Department of Strategic Programs, EPOC, National Cancer Center

tumor accumulation via active targeting, attenuating antitumor efficacy, whereas SA successfully maintains tumor targeting and antitumor activity. In alpha-radioimmunotherapy, active targeting significantly increases tumor accumulation of ^{211}At.

In conclusion, SA-dependent protection that maintains tumor targeting and *in vivo* antitumor effect will facilitate the clinical application of ^{211}At-radioimmunotherapy.

References

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