Influence of antibody stabilization with sodium ascorbate on radioimmunotherapy with an ^{211}At-conjugated anti-tissue factor antibody†

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Alpha radiation is characterized by higher linear transfer compared to other types of ionizing radiation and a range of 50–100 μm in tissue. Therefore, the selective tumor accumulation of alpha emitters exerts potent antitumor effects without serious toxicity against normal cells adjacent to the tumor. Astatine-211 (^{211}At) is an alpha emitter, its high production yield is sufficient to prepare ^{211}At-labeled radiopharmaceuticals for administration at clinical doses.

Tissue factor (TF), a transmembrane glycoprotein initiating the extrinsic blood coagulation cascade, is overexpressed in tumors such as gastric cancer, pancreatic cancer, and malignant gliomas.[1,2)]

Immunoglobulin G (IgG) selectively accumulate in tumor via the enhanced permeability and retention (EPR) effect.[3)] In addition, the antigen-antibody reaction enhances the tumor accumulation of antibodies.[2)] Therefore, we focused on applying anti-TF monoclonal antibodies (mAbs) established by us to armed antibodies such as antibody-drug conjugate (ADC)[4–6)] and antibody labeled with therapeutic radionuclides.

We synthesized ^{211}At in the ^{209}Bi(α, $2n$)^{211}At reaction using the RIKEN azimuthally varying field (AVF) cyclotron, and we labeled an anti-TF mAb with the radionuclide as previously reported.[7)]

To evaluate ^{211}At-conjugaed anti-TF mAb, we performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and flow cytometry analyses. In the SDS-PAGE analysis, ^{211}At-conjugated anti-TF mAb was smeared, which was in contrast to band patterns in ^{211}At-unlabeled anti-TF mAbs. Consequently, flow cytometry analysis revealed that the binding activity of the astatinated mAb was disturbed compared with ^{211}At-unlabeled mAbs. These findings suggest that anti-TF mAb was denatured by the ^{211}At-induced radiochemical reaction. Then, to protect anti-TF mAb from denaturation, we purified astatinated mAbs using an elution buffer containing sodium ascorbate (SA), which is a free radical scavenger. In the SDS-PAGE analysis, band patterns were demonstrated in ^{211}At-conjugated anti-TF mAbs eluted in phosphate-buffered saline (PBS) containing 0.6 or 1.2% SA and ^{211}At-unlabeled mAbs. Consequently, the binding activities of the astatinated anti-TF mAbs in the SA solution were comparable to those of ^{211}At-unlabeled mAbs.

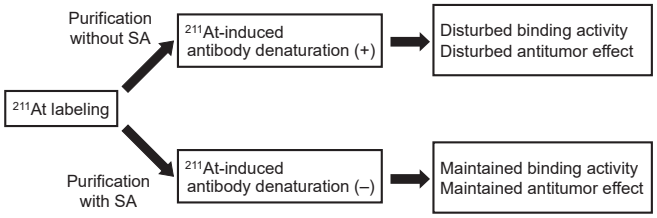

Fig. 1. Summary of ^{211}At-induced antibody denaturation and protective effect of sodium ascorbate.

^{211}At-conjugated anti-TF mAbs stabilized with SA exerted greater cytocidal effects on gastric cancer cells than the astatinated mAb eluted in PBS. Similar to ADC, the cytotoxicities of the stabilized immunoconjugates depended on the level of TF in the cancer cell.

To evaluate *in vivo* toxicities, we observed the body weight loss in mice administered SA, ^{211}At-conjugated anti-TF mAb, or free ^{211}At. Although body weight loss was observed in mice administered PBS containing 1.2% SA, the loss was transient and the radioprotectant seemed tolerable. Body weight loss after the administration of the astatinated anti-TF mAb in PBS containing 1.2% SA was milder than free ^{211}At dissolved in 1.2% SA solution.

^{211}At-conjugated anti-TF mAb eluted in PBS containing 1.2% SA showed significantly greater antitumor effects in a high TF-expressing gastric cancer xenograft model than the non-stabilized immunoconjugate. Similar to ADC, the antitumor effect of the astatinated anti-TF mAb in 1.2% SA soution depended on the TF expression on the cell membrane of cancer cells.

In summary, SA protected the astatinated anti-TF mAb from ^{211}At-induced antibody denaturation, which resulted in the maintained binding and antitumor activities of the immunoconjugate (Fig. 1). Without intolerable side effects, ^{211}At-conjugated anti-TF mAb eluted in PBS containing 1.2% SA showed potent antitumor effects in gastric cancer xenograft models dependent on the level of TF on the cancer cell membrane.

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