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Anti-Human Epidermal Growth Factor Receptor 2 Monoclonal Antibody H_2Mab-41 Exerts Antitumor Activity in a Mouse Xenograft Model of Colon Cancer

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The expression of human epidermal growth factor receptor 2 (HER2) has been reported to be overexpressed in several cancers, such as breast, lung, gastric, pancreatic, and colorectal cancers, and be associated with poor clinical outcomes. Trastuzumab, a humanized anti-HER2 antibody, provides significant survival benefits for patients with HER2-overexpressing breast cancers and gastric cancers. In this study, we developed a novel anti-HER2 monoclonal antibody (mAb), H_2Mab-41 (IgG_{2b}, kappa), and the antitumor activity of H_2Mab-41 was investigated using mouse xenograft models. Caco-2 cells (human colon cancer cell line), which expresses HER2, were subcutaneously implanted into the flanks of nude mice. H_2Mab-41 and control mouse IgG were injected three times into the peritoneal cavity of mice. H_2Mab-41 significantly reduced tumor development of Caco-2 xenograft in comparison with the control mouse IgG on days 5, 8, 11, 15, and 19. Taken together, these results suggest that H_2Mab-41 is useful for antibody therapy against HER2-expressing colon cancers.

Keywords: HER2, monoclonal antibody, antitumor activity, colon cancer

Introduction

TRASTUZUMAB AND PERTUZUMAB, humanized anti-human epidermal growth factor receptor 2 (HER2) monoclonal antibodies (mAbs), and trastuzumab emtansine, an antibody–drug conjugate (ADC), have been approved for the treatment of HER2-positive breast cancer.[1–3] Trastuzumab treatment has resulted in significant survival benefits for patients with metastatic HER2-positive breast cancer,[4] although HER2 is associated with poor clinical outcomes.[5,6] Furthermore, the combination of pertuzumab and trastuzumab with chemotherapy has led to significant improvements in overall survival compared with trastuzumab alone plus chemotherapy.[7] HER2 overexpression has been also reported in gastric cancers,[8] pancreatic cancers,[9] lung cancers,[10] and colorectal cancers.[11] In this study, we developed a novel anti-HER2 mAb H_2Mab-41, and investigated its antitumor activity against mouse xenograft models of human colon cancer.

Materials and Methods

Cell lines

P3U1, LN229, and Caco-2 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). LN229/HER2 cells were established in our previous study.[12] P3U1 was cultured in RPMI 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan), and LN229, LN229/HER2, and Caco-2 were cultured in the Dulbecco's modified Eagle's medium (DMEM) medium (Nacalai Tesque, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Inc., Waltham, MA), 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 25 μg/mL of amphotericin B (Nacalai Tesque, Inc.) at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air.

Hybridoma production

Female 4-week-old BALB/c mice were purchased from CLEA Japan (Tokyo, Japan). Animals were housed under specific pathogen-free conditions. The Animal Care and Use Committee of Tohoku University approved all the animal experiments described in this study. Anti-HER2 hybridomas were produced, as described previously.[12] In brief, BALB/c mice were immunized using intraperitoneal (i.p.) injections of 100 μg of recombinant HER2-extracellular domain together with Imject Alum (Thermo Fisher Scientific, Inc.). After several additional immunizations, a booster injection was intraperitoneally administered 2 days before harvesting

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spleen cells. Spleen cells were then fused with P3U1 cells using PEG1500 (Roche Diagnostics, Indianapolis, IN). The resulting hybridomas were grown in RPMI medium supplemented with hypoxanthine, aminopterin, and thymidine selection medium supplement (Thermo Fisher Scientific, Inc.). Culture supernatants were screened using enzyme-linked immunosorbent assay with recombinant HER2-extracellular domain. mAbs were purified from the supernatants of hybridomas, cultured in Hybridoma-SFM medium (Thermo Fisher Scientific, Inc.) using Protein G Sepharose 4 Fast Flow (GE Healthcare UK Ltd., Buckinghamshire, England).

Flow cytometry

Cells were harvested by brief exposure to 0.25% trypsin/1-mM ethylenediaminetetraacetic acid (Nacalai Tesque, Inc.). After washing with 0.1% bovine serum albumin/phosphate-buffered saline (PBS), the cells were treated with 1 μg/mL of anti-HER2 (H_2Mab-41 and H_2Mab-139) for 30 minutes at 4°C and subsequently with Alexa Fluor 488-conjugated anti-mouse IgG (1:1000; Cell Signaling Technology, Inc., Danvers, MA). Fluorescence data were collected using EC800 Cell Analyzers (Sony Corp., Tokyo, Japan).

Antitumor activity of H_2Mab-41

Female BALB/c nude mice (6-week old) were purchased from Charles River (Kanagawa, Japan) and used in experiments when they were 10 weeks old. Caco-2 (0.3 mL of 1.33×10^8/mL in DMEM) were mixed with 0.5 mL of BD Matrigel Matrix Growth Factor Reduced (BD Biosciences, San Jose, CA). A 100-μL suspension (containing 5×10^6 cells) was injected subcutaneously into the left flanks of nude mice. After day 1, 100 μg of H_2Mab-41 and control mouse IgG (Sigma-Aldrich Corp., St. Louis, MO) in 100 μL PBS were injected into the peritoneal cavity of each mouse. Additional antibodies were then injected on days 8 and 15. The tumor diameter and volume were determined as previously described.[13] The mice were euthanized 19 days after cell implantation. All data were expressed as mean ± SEM. Statistical analysis was performed using the Tukey–Kramer test. $p < 0.05$ was considered to be statistically significant.

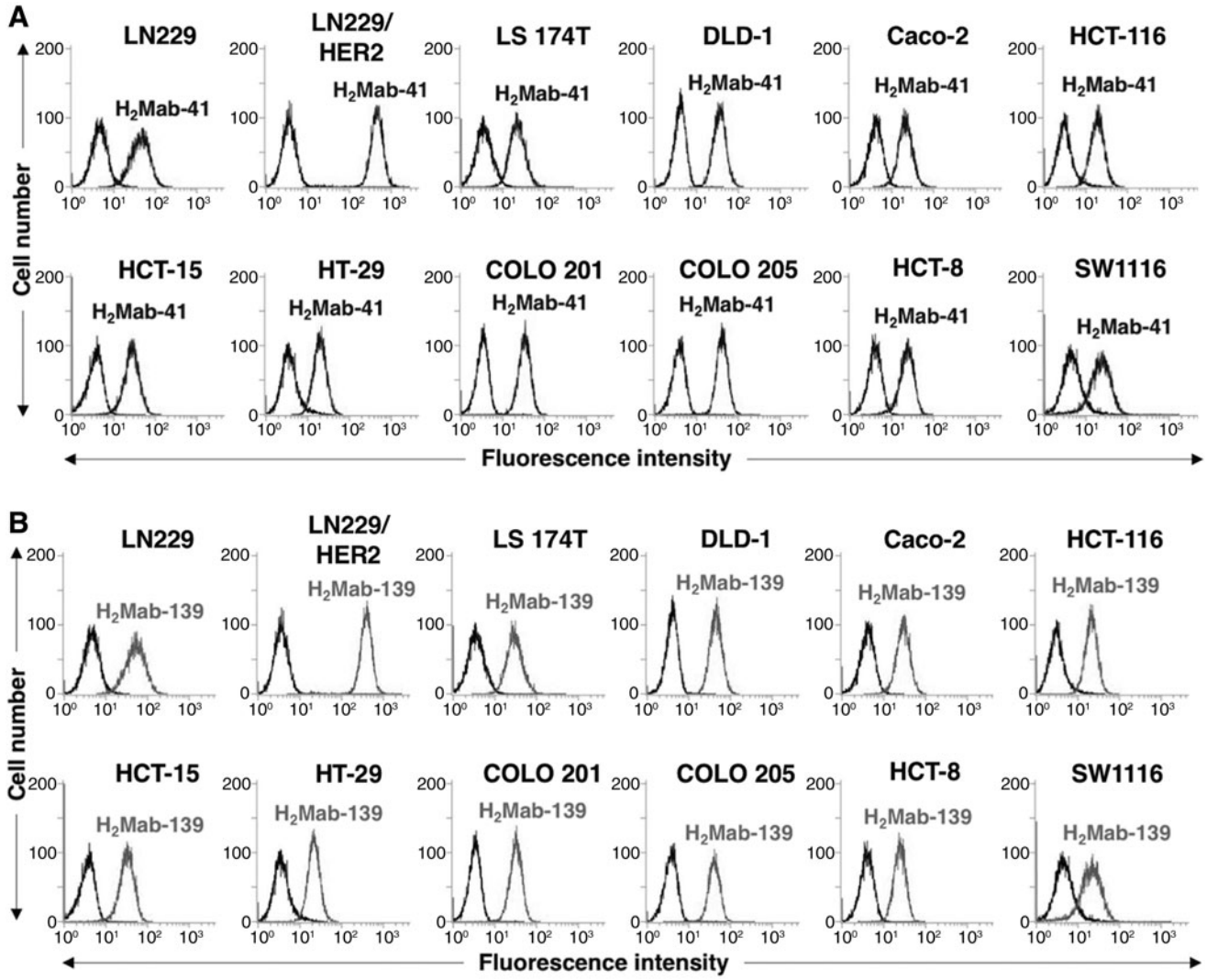

FIG. 1. Flow cytometric analysis of H_2Mab-41 against colon cancer cell lines. Cells were treated with 1 μg/mL of H_2Mab-41 **(A)** and H_2Mab-139 **(B)**, followed by Alexa Fluor 488-conjugated anti-mouse IgG; left line, negative control.

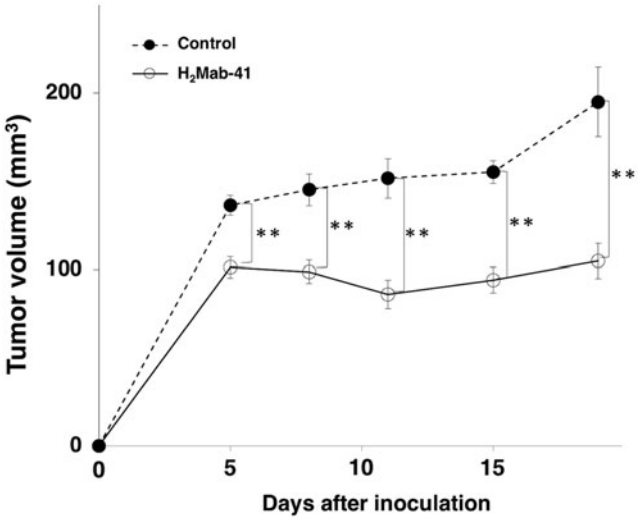

FIG. 2. Evaluation of antitumor activity (tumor volume) of H_2Mab-41 against Caco-2 xenograft model. Tumor volume of Caco-2 xenografts. Caco-2 cells were injected subcutaneously into female nude mice. The indicated antibodies (100 μg/day; 5 mg/kg) were administered intraperitoneally on days 1, 8, and 15 after cell inoculation. The tumor volume was measured at the indicated time points. The values are presented as mean ± SEM. $^{**}p < 0.01$, Tukey–Kramer's test.

Results

In this study, we immunized one mouse with the recombinant extracellular domain of HER2,[14] which was purified using MAP tag system.[15] Flow cytometry was performed to check reactions with LN229 (a glioblastoma cell line) and HER2-overexpressing LN229 (LN229/HER2) cells (data not shown). A stronger reaction against LN229/HER2 was needed compared with LN229 because LN229 cells endogenously express HER2. We obtained one clone H_2Mab-41 of IgG_{2a} subclass although almost all mAbs were determined to be a mouse IgG_1 subclass.

We first characterized H_2Mab-41 in flow cytometry. H_2Mab-41 reacted with LN229/HER2 cells, weakly reacted with LN299 cells (Fig. 1A), indicating that H_2Mab-41 is specific for HER2. As a positive control, H_2Mab-139 also reacted in the same pattern (Fig. 1B). Furthermore, H_2Mab-41 recognized endogenous HER2 in colon cancer cell lines, such as Caco-2, HCT-116, HCT-15, HT-29, LS 174T, COLO 201, COLO 205, HCT-8, SW1116, and DLD-1 (Fig. 1A) in the same pattern with H_2Mab-139 (Fig. 1B).

To study the antitumor activity of H_2Mab-41 on cell growth *in vivo*, Caco-2 cells were subcutaneously implanted into the flanks of nude mice. H_2Mab-41 and control mouse IgG were injected three times (on days 1, 8, and 15 after cell injections) into the peritoneal cavity of mice. Tumor formation was observed in mice from the control and H_2Mab-41-treated groups in Caco-2 xenograft models. H_2Mab-41 significantly reduced the tumor development of Caco-2 xenograft in comparison with that in control mouse IgG on days 5, 8, 11, 15, and 19 (Fig. 2). The resected tumors of Caco-2 xenografts are depicted in Figure 3A. The tumor weight of

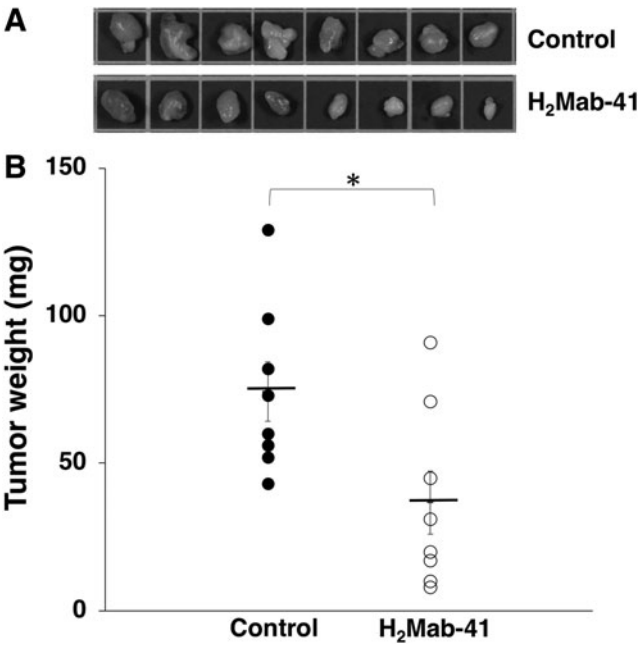

FIG. 3. Evaluation of antitumor activity (tumor weight) of H_2Mab-41 against Caco-2 xenograft model. **(A)** Resected tumors of Caco-2 xenografts. **(B)** Tumor weight of Caco-2 xenografts (day 19). $^{*}p < 0.05$, Tukey–Kramer's test.

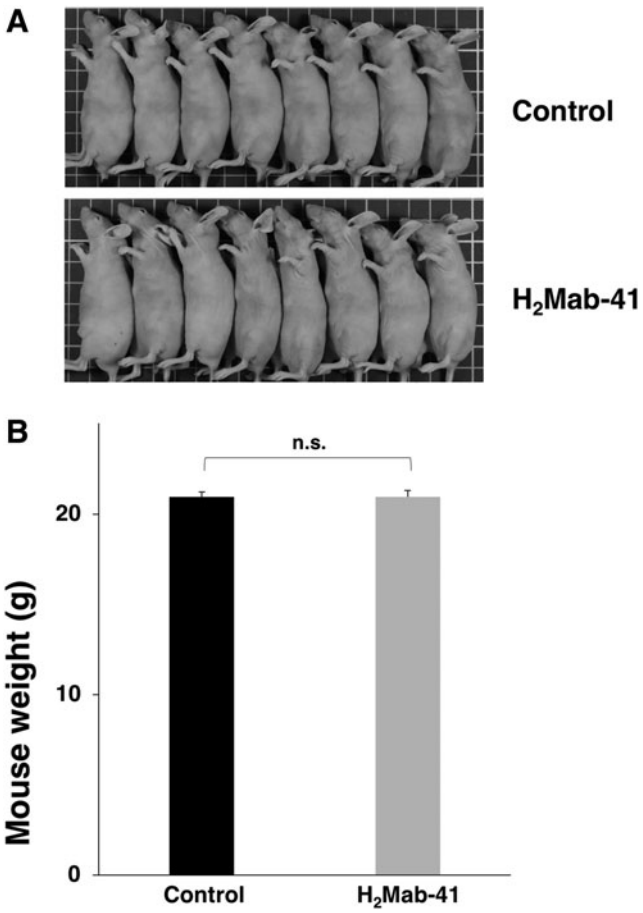

FIG. 4. Evaluation of antitumor activity (body weight) of H_2Mab-41 against Caco-2 xenograft model. **(A)** Caco-2 xenograft mice models on day 19. **(B)** Body weight of Caco-2 xenografts (day 19). The values are presented as mean ± SEM. n.s., not significant.

mice in H_2Mab-41-treated group was significantly lower than that in the control mouse IgG group in Caco-2 xenograft models (Fig. 3B). Caco-2 xenograft mice models on day 19 are shown in Figure 4A. Body weight was not significantly different among the two groups in the Caco-2 xenograft models (Fig. 4B).

Discussion

Recently, trastuzumab deruxtecan (also known as DS-8201) was reported to be an ADC, which comprised trastuzumab, a novel enzyme-cleavable linker, and a topoisomerase I inhibitor payload.[16] Of interest, trastuzumab deruxtecan showed antitumor activity, even in low HER2-expressing tumors. Trastuzumab deruxtecan has several innovative features: (i) a highly potent novel payload with a high drug-to-antibody ratio, (ii) good homogeneity, (iii) a tumor-selective cleavable linker, (iv) a stable linker-payload in circulation, and (v) a short systemic half-life cytotoxic agent *in vivo*.[17] Furthermore, the released cytotoxic payload could exert a bystander effect.[17] Although ADC technology is very critical technology, the development of more functional mAbs might be still necessary.

Recently, we developed several anti-HER2 mAbs, such as H_2Mab-77,[12] H_2Mab-119,[18] and H_2Mab-139[14] using CasMab technology.[19] Those anti-HER2 mAbs are useful for flow cytometry, Western blot, and immunohistochemical analyses. However, those three mAbs do not possess antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) because the subclass of those mAbs is mouse IgG_1. Among the mouse IgG subclasses, IgG_{2a}[20] and IgG_{2b}[21] are known to possess ADCC and CDC. Then, we successfully screened H_2Mab-41 of IgG_{2b} subclass. Unfortunately, H_2Mab-41 did not react with HER2 in Western blot and immunohistochemical analyses (data not shown). In contrast, H_2Mab-41 exerted antitumor activity against a colon cancer xenograft, indicating that H_2Mab-41 is applicable for antibody therapy against human colon cancers expressing HER2. Further studies on antitumor activities against HER2-expressing xenografts are, therefore, necessary to obtain a more detailed understanding of antibody therapy against HER2.

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Author Disclosure Statement

No competing financial interests exist.

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