MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 36, Number 1, 2017

DOI: 10.1089/mab.2016.0045

Antiglycopeptide Mouse Monoclonal Antibody LpMab-21 Exerts Antitumor Activity Against Human Podoplanin Through Antibody-Dependent Cellular Cytotoxicity and Complement-Dependent Cytotoxicity

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The interaction between podoplanin (PDPN) and C-type lectin-like receptor 2 (CLEC-2) is involved in tumor malignancy. We have established many monoclonal antibodies (mAbs) against human podoplanin using the cancer-specific mAb (CasMab) technology. LpMab-21, one of the mouse antipodoplanin mAbs, is of the IgG_{2a} subclass, and its minimum epitope was determined to be Thr76–Arg79 of the human podoplanin. Importantly, sialic acid is linked to Thr76; therefore, LpMab-21 is an antiglycopeptide mAb (GpMab). In this study, we investigated whether LpMab-21 shows antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) against human podoplanin-expressing cancer cell lines *in vitro* and also studied its antitumor activities using a xenograft model. LpMab-21 showed high ADCC and CDC activities against not only podoplanin-expressing Chinese hamster ovary cells but also LN319 glioblastoma cells and PC-10 lung cancer cells, both of which endogenously express podoplanin. Furthermore, LpMab-21 decreased tumor growth *in vivo*, indicating that LpMab-21 could be useful for antibody therapy against human podoplanin-expressing cancers.

Keywords: podoplanin, monoclonal antibody, ADCC, CDC, antitumor activity

Introduction

Podoplanin (PDPN) is expressed in many tumors, such as brain tumors, esophageal cancers, lung cancers, malignant mesotheliomas, osteosarcomas, fibrosarcomas, testicular cancers, and bladder cancers.[1–15] Importantly, the expression of human podoplanin in cancer-associated fibroblasts contributes to poor prognosis.[16–21] C-type lectin-like receptor 2 (CLEC-2) is an endogenous receptor of human podoplanin.[22,23] It binds to human podoplanin through residues Glu47 and Asp48 within its platelet aggregation-stimulating domain, and it also binds to the α-2,6-linked sialic acid linked to Thr52.[24]

Although many antihuman podoplanin monoclonal antibodies (mAbs) are commercially available, almost all the mAbs react with the N-terminus of human podoplanin.[6,25–29] In contrast, we have used the cancer-specific mAb (CasMab) technology to produce antiglycopeptide mAbs (GpMabs) against human podoplanin.[30–39] Recently, we have successfully developed a novel antihuman podoplanin mAb, LpMab-21, which recognizes a sialylated glycopeptide epitope.[40] LpMab-21 is one of the GpMabs, but not a CasMab.

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Importantly, LpMab-21 is the first mouse antihuman podoplanin mAb of the IgG_{2a} subclass, which was generated using the CasMab technology.[30–36,38,39]

Results and Discussion

We have produced several antihuman podoplanin mAbs using the CasMab technology; however, the isotypes of the mAbs are IgG_1 (seven clones) and IgG_3 (one clone), which do not induce antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). The applications of mouse IgG_3 mAbs are limited because they often aggregate.[41] In this case, we have had to convert them to human IgG_1 to investigate whether these mAbs cause ADCC and CDC.[33] In contrast, the mouse IgG_{2a} subclass can induce ADCC and CDC; therefore, we investigated whether LpMab-21 can induce ADCC and CDC against human podoplanin-expressing cancer cell lines. As shown in Figure 1A, LpMab-21 demonstrated ADCC against LN319, the Chinese hamster ovary (CHO)/human podoplanin, and PC-10 cell lines, whereas it did not induce ADCC against human podoplanin-negative parental CHO cells. Similarly, LpMab-21 induced CDC against LN319 and PC-10 cell lines (Fig. 1B). We previously demonstrated that LN319 expresses human podoplanin at a higher level than does PC-10[30]; therefore, ADCC and CDC might depend on the human podoplanin expression levels in these cell lines.

To investigate the antitumor activity of LpMab-21 on primary tumor growth *in vivo*, CHO/human podoplanin cells were subcutaneously implanted into flanks of nude mice. LpMab-21 or control mouse IgG (clone PMab-2)[42] was injected into the peritoneal cavity of the mice once weekly for 4 weeks ($n=6$ each). PMab-2 was raised against rat podoplanin and was shown to not cross-react with human podoplanin.[42] Tumor formation was observed in five mice from the control group (tumor incidence on day 35: 83.3%, 5/6; Supplementary Fig. S1). In contrast, LpMab-21 dramatically reduced the tumor development (tumor incidence on day 35: 50%, 3/6; Supplementary Fig. S1). The tumor volume was significantly reduced by LpMab-21 treatment (Fig. 2). These results indicate that administration of LpMab-21 inhibited the primary tumor growth of CHO/human podoplanin cells. In our previous study, only CDC of a human–mouse chimeric anti-PDPN mAb could show antitumor activity because human NK cell was not added in this xenograft model.[43] In contrast, we also showed that ADCC of a human–mouse chimeric anti-PDPN mAb is more important than CDC in another study.[9] Therefore, we think that both ADCC and CDC are important to induce antitumor activity in the xenograft model.

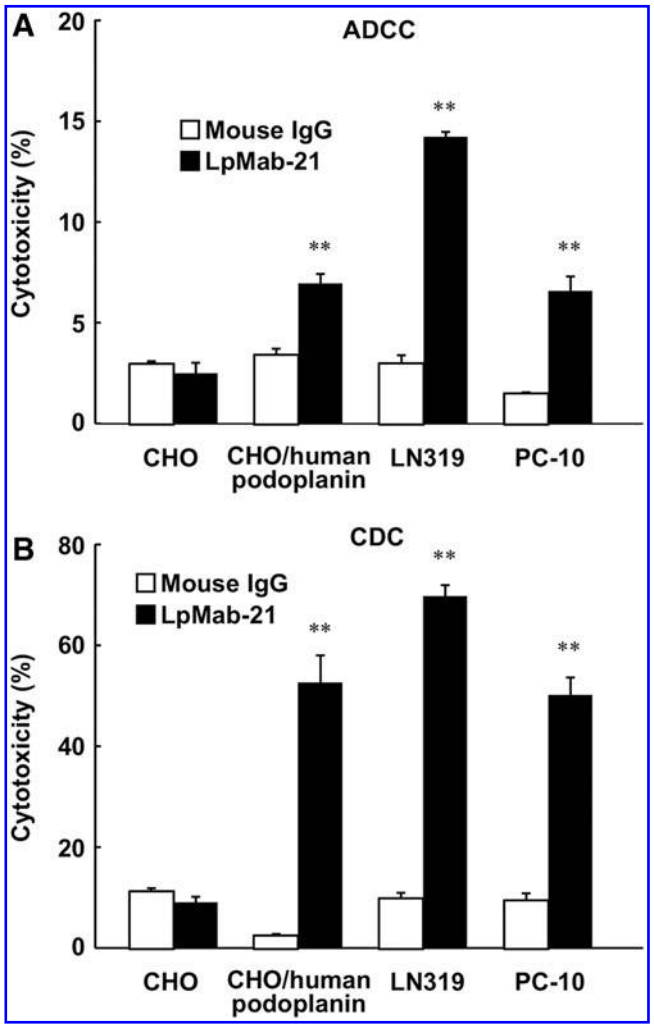

FIG. 1. Antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) of LpMab-21 against human podoplanin-expressing cell lines. **(A)** ADCC activity of LpMab-21. Mouse splenocytes were harvested from severe combined immunodeficiency mouse spleens. Spleens were homogenized in RPMI 1640 and centrifuged. To deplete red blood cells, the cell pellet was suspended in red blood cell lysis buffer (Sigma–Aldrich). After washing and resuspension in cRPMI1640, splenocytes were used as effector cells. ADCC was determined using the ^{51}Cr release assay. Target cells were incubated with 0.1 μCi of [^{51}Cr]sodium chromate at 37°C for 1 hour. After washing with cRPMI1640 three times, ^{51}Cr-labeled target cells were placed in 96-well plates in triplicate. Effector cells and LpMab-21 or control mouse IgG were added to the plates. After 6 hours of incubation, ^{51}Cr release was measured in the supernatant (100 μL) from each well using a gamma counter (PerkinElmer). The percentage of cytotoxicity was calculated using the following formula: specific lysis $(\%) = (E - S)/(M - S) \times 100$, where E is the ^{51}Cr release in the test sample, S is the spontaneous release, and M is the maximum release. Statistical significance of the differences in *in vitro* data was analyzed by the standard Student's *t*-test. In this study, *p* values of <0.05 were considered statistically significant in all experiments. $^{**}p < 0.01$ **(B)** CDC was evaluated with the ^{51}Cr release assay. Target cells were incubated with [^{51}Cr]sodium chromate (0.1 μCi) for 1 hour at 37°C. The cells were then washed with cRPMI1640. The ^{51}Cr-labeled cells were incubated with a baby rabbit complement (Cedarlane) at a dilution of 1:32 (Chinese hamster ovary [CHO] and CHO/human podoplanin) or 1:4 (LN319 and PC-10) in the presence of LpMab-21 (1 μg/mL) or control mouse IgG (1 μg/mL) for 3 hours (CHO and CHO/human podoplanin) or 6 hours (LN319 and PC-10) in 96-well plates. After the incubation, the ^{51}Cr radioactivity was measured in the supernatants using a gamma counter. The percentage of cytotoxicity was calculated as described previously. $^{**}p < 0.01$.

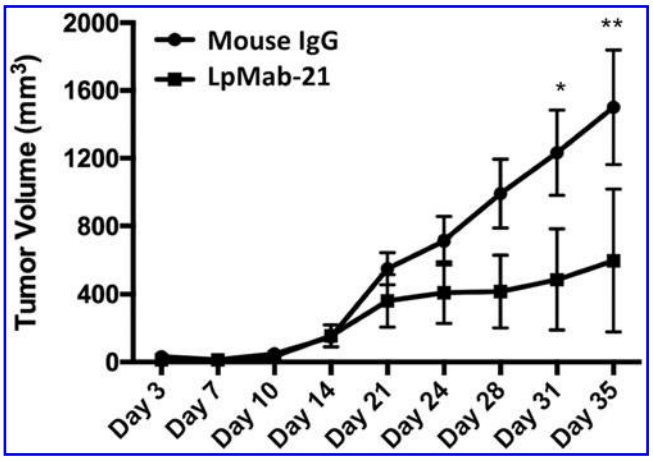

FIG. 2. Antitumor effects of LpMab-21 on primary tumor development. CHO/human podoplanin cells (3×10^6 cells/100 μL) were subcutaneously inoculated into BALB/c nude mice. After 1 day, 100 μg of LpMab-21 or control mouse IgG (clone PMab-2) was injected into the peritoneal cavity of the mice. The antibodies were injected once weekly for 4 weeks (control group: $n=6$; LpMab-21 group: $n=6$). The tumor diameter was measured at intervals of 3 to 4 days and was calculated using the following formula: tumor volume $= W^2 \times L/2$, where W is short diameter and L is long diameter. $*p<0.05$; $**p<0.01$ with two-way analysis of variance.

Taken together, LpMab-21 could be useful for antibody therapy against human podoplanin-expressing cancers. Our developed CasMabs as well as LpMab-21 could be applied to the novel antitumor reagents, including T cells and viruses,[44] to give strict specificity against tumor cells.

Acknowledgments

This work was supported, in part, by project for utilizing glycans in the development of innovative drug discovery technologies from Japan Agency for Medical Research and development, AMED (Y.K.), by the Basic Science and Platform Technology Program for Innovative Biological Medicine from AMED (Y.K.), by the Platform for Drug Discovery, Informatics, and Structural Life Science (PDIS) from AMED (Y.K., T.M.), by Translational Research Network Program from AMED (Y.K.), by JSPS KAKENHI Grant Number 26440019 (M.K.K.) and 16K10748 (Y.K.), and by the Regional Innovation Strategy Support Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (Y.K.). This work was performed, in part, under the Cooperative Research Program of Institute for Protein Research, Osaka University, CR-16–05, and by the Grant for Joint Research Project of the Institute of Medical Science, the University of Tokyo. The authors would like to thank Enago (www.enago.jp) for the English language review.

Author Disclosure Statement

No competing financial interests exist.

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Received: November 14, 2016
Accepted: December 19, 2016