MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 40, Number 4, 2021
© Mary Ann Liebert, Inc.
DOI: 10.1089/mab.2021.0023

Development of a Novel Epitope Mapping System: RIEDL Insertion for Epitope Mapping Method

Teizo Asano,[1] Mika K. Kaneko,[1] and Yukinari Kato[1,2,i]

To clarify the binding region of monoclonal antibodies (mAbs) to target molecules, it is very essential to understand the pharmacological function of each mAb. Although deletion mutants and point mutants are usefully utilized for epitope mapping, we often experience the difficulty of determining the mAb epitope against membrane proteins. We aimed to develop a novel method to determine the binding region of mAbs using epitope tag system. We first checked the reactivity of an anti-CD44 mAb (C_{44}Mab-5) to several deletion mutants of CD44. We then employed the RIEDL tag system (''RIEDL'' peptide and LpMab-7 mAb). We inserted the ''RIEDL'' peptide into the CD44 protein from the 21st to 41st amino acid (AA). The transfectants produced were stained by LpMab-7 and C_{44}Mab-5 in flow cytometry. C_{44}Mab-5 did not react with 30th–361st AA of the deletion mutant of CD44. Furthermore, the reaction of C_{44}Mab-5 to RIEDL tag-inserted CD44 from 25th to 36th AA was lost, although LpMab-7 detected most of the RIEDL tag-inserted CD44 from 21st to 41st AA. The epitope of C_{44}Mab-5 for CD44 was determined to be the peptide from 25th to 36th AA of CD44 using RIEDL insertion for epitope mapping (REMAP) method. The REMAP method might be useful for determining the critical epitope of functional mAbs against many target molecules.

Keywords: CD44, C44Mab-5, epitope mapping, monoclonal antibody, RIEDL tag

Downloaded by Yukinari Kato from www.liebertpub.com at 08/23/21. For personal use only.

Introduction

An epitope is a part of an antigen, which is recognized by antibodies. In general, the epitope for monoclonal antibodies (mAbs) consists of several amino acids (AAs); therefore, mAbs often might cross-react with unexpected proteins. Identification of the mAbs epitope is important to avoid unexpected cross-reactivity and is helpful for the development of antibody drug.

Several epitope-determining methods have been reported,[1] including X-ray cocrystallography,[2] array-based oligopeptide scanning,[3] and site-directed mutagenesis mapping.[4,5] Although X-ray cocrystallography can clearly determine the epitope because this method allows direct visualization of the interaction between the antigen and antibody, the crystallization of the antigen–antibody complex usually takes time and costs a lot. In contrast, the array-based oligopeptide scanning and site-directed mutagenesis mapping can easily determine linear epitopes, but is not appropriate for determining conformational epitopes. Therefore, development of simple and efficient methods for determining conformational epitopes is desired.

We developed a mAb against human CD44 (clone C_{44}Mab-5) by immunizing mice with CD44-overexpressed cells. However, the epitope for C_{44}Mab-5 has not been determined using array-based oligopeptide scanning or site-directed mutagenesis mapping. CD44 is related to development, metastasis, and invasion of tumor[6–9]; therefore, CD44 has been studied as a therapeutic target and biomarker in several cancers.[10] C_{44}Mab-5 is applicable for flow cytometry and immunohistochemistry.[11] C_{44}Mab-5 can recognize not only CD44s, but also CD44 variants.

Recently, we converted the mouse IgG_1 subclass antibody C_{44}Mab-5 into an IgG_{2a} subclass antibody and further produced a defucosylated version (5-mG_{2a}-f). The 5-mG_{2a}-f showed antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) against oral squamous cell carcinoma.[12] Therefore, 5-mG_{2a}-f may be a useful therapy for patients with CD44-expressing oral cancer, and determination of the epitope for C_{44}Mab-5 is desired.

In this study, we aimed to develop a novel epitope mapping method that is a simple and an efficient method for determining the conformational epitopes. Then, we tried to

[1]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan.
[2]Department of Molecular Pharmacology, Tohoku University Graduate School of Medicine, Sendai, Japan.
[i]ORCID ID (https://orcid.org/0000-0001-5385-8201).

Downloaded by Yukinari Kato from www.liebertpub.com at 08/23/21. For personal use only.

determine the epitope region for C_{44}Mab-5 on CD44 using the novel epitope mapping system: RIEDL insertion for epitope mapping (REMAP) method using RIEDL tag system.[13]

Materials and Methods

Plasmid preparation

CD44s open reading frame (ORF) was amplified from LN229 cDNA using HotStar HiFidelity Polymerase Kit (Qiagen, Inc., Hilden, Germany). CD44v3-10 ORF was provided by RIKEN BRC through the National Bio-Resource Project of MEXT, Japan. CD44s ORF and CD44v3-10 ORF without original signal sequence (1st–20th AAs) were subcloned into pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), and a IL-2 signal sequence (MYRMQLLSCIALSLALVTNS) and PA16 tag (GLEGGVAMPGAEDDVV),[14,15] which is recognized by an anti-PA16 tag mAb (NZ-1),[16] were added at the N-terminal. The deletion mutants of CD44s or CD44v3-10 were performed using HotStar HiFidelity Polymerase Kit with oligonucleotides containing the desired mutations. Substitutions of an AA to alanine in CD44s sequence were conducted by QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Inc., Santa Clara, CA). Insertions of RIEDL tag in CD44s sequence were performed using HotStar HiFidelity Polymerase Kit with oligonucleotides containing RIEDL tag insertions at the desired position. For example, we produced Gln21_RIEDL_Ile22 (Q21_R*_I22) by inserting the RIEDL sequence between Gln21 and Ile22 of CD44s. Polymerase chain reaction fragments bearing the desired mutations were inserted into pCAG-Ble vector using In-Fusion HD Cloning Kit (Takara Bio, Inc., Shiga, Japan). The RIEDL tag insertion mutants produced are Q21_R*_I22, I22_R*_D23, D23_R*_L24, L24_R*_N25, N25_R*_I26, I26_R*_T27, T27_R*_C28, C28_R*_R29, R29_R*_F30, F30_R*_A31, A31_R*_G32, G32_R*_V33, V33_R*_F34, F34_R*_H35, H35_R*_V36, V36_R*_E37, E37_R*_K38, K38_R*_N39, N39_R*_G40, and G40_R*_R41.

Cell lines

Chinese hamster ovary (CHO)-K1 was obtained from the America Type Culture Collection (ATCC, Manassas, VA). CD44 mutation plasmids were transfected into CHO-K1 cells using Neon Transfection System (Thermo Fisher Scientific, Inc., Waltham, MA) and stable transfectants were sorted by PA16 tag using a cell sorter (SH800; Sony Corp., Tokyo, Japan). The CHO-K1 cells and transfectants were cultured in RPMI 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan) supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Inc.), 100 U/mL of penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Nacalai Tesque, Inc.) at 37°C in a humidified atmosphere containing 5% CO_2. The stable transfectants were cultivated in a medium containing 0.5 mg/mL Zeocin (InvivoGen, San Diego, CA) for selection.

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 in phosphate-buffered saline, cells were treated with primary mAbs (1 μg/mL) for 30 minutes at 4°C and subsequently with Alexa Fluor 488-conjugated antimouse IgG (1:1000; Cell Signaling Technology, Inc., Danvers, MA). Fluorescence data were collected using EC800 Cell Analyzer (Sony Corp.).

Result

Determination of C_{44}Mab-5 epitope using deletion mutants of CD44s

The epitope of C_{44}Mab-5 might exist at the extracellular region of CD44s because C_{44}Mab-5 was developed using Cell-Based Immunization and Screening method.[17] First, we produced N-terminal deletion mutants (dN30, dN40, dN50, dN60, dN70, and dN79) with PA16 tag at their N-terminal and investigated the reactivity between C_{44}Mab-5 and each deletion mutant by flow cytometry analysis. The results showed that C_{44}Mab-5 recognized only CD44s and CD44v3-10, but did not recognize all the deletion mutants (dN30, dN40, dN50, dN60, dN70, and dN79) (Fig. 1A). In contrast, all deletion mutants were detected by an anti-PA16 tag mAb, NZ-1 (Fig. 1B). These results show that the N-terminus of the C_{44}Mab-5 epitope exists between 21st and 29th AAs of CD44s (Fig. 1C).

Determination of C_{44}Mab-5 epitope using point mutants of CD44s

Next, we produced constructs of 20 point mutants within CD44s (Q21A, I22A, D23A, L24A, N25A, I26A, T27A, C28A, R29A, F30A, A31G, G32A, V33A, F34A, H35A, V36A, E37A, K38A, N39A, and G40A) and performed flow cytometry analysis using transient transfectants. All CD44s point mutants were recognized not only by NZ-1, but also by C_{44}Mab-5 (Supplementary Fig. S1). These results indicate that the epitope of C_{44}Mab-5 will be a conformational epitope, since we could not determine the crucial epitope by alanine scanning.

Determination of C_{44}Mab-5 epitope using REMAP method

To further investigate the critical AAs of conformational epitope of C_{44}Mab-5, we conducted a tag insertion scanning, which is named as REMAP method. In this method, we used a RIEDL tag system.[13] RIEDL tag system consists of five AAs peptide (RIEDL tag) and an anti-RIEDL tag mAb (clone LpMab-7). C_{44}Mab-5 can bind to CD44s when RIEDL tag is inserted into any region, which is independent of the C_{44}Mab-5 epitope (Fig. 2A, upper panel). In contrast, CD44s will not be detected by C_{44}Mab-5 when the conformation of the C_{44}Mab-5 epitope is disrupted by RIEDL insertion (Fig. 2A, lower panel). We constructed 20 mutants of CD44s, in which RIEDL tag was inserted into the expected epitope region within CD44s (Fig. 2B). Results of the flow cytometry analysis showed that C_{44}Mab-5 did not detect 11 mutants (N25_R*_I26, I26_R*_T27, T27_R*_C28, C28_R*_R29, R29_R*_F30, F30_R*_A31, A31_R*_G32, G32_R*_V33, V33_R*_F34, F34_R*_H35, H35_R*_V36), but weakly detected L24_R*_N25 (Fig. 3A), indicating that C_{44}Mab-5 binds to CD44s through 12 AAs (from Asn25 to Val36). As a

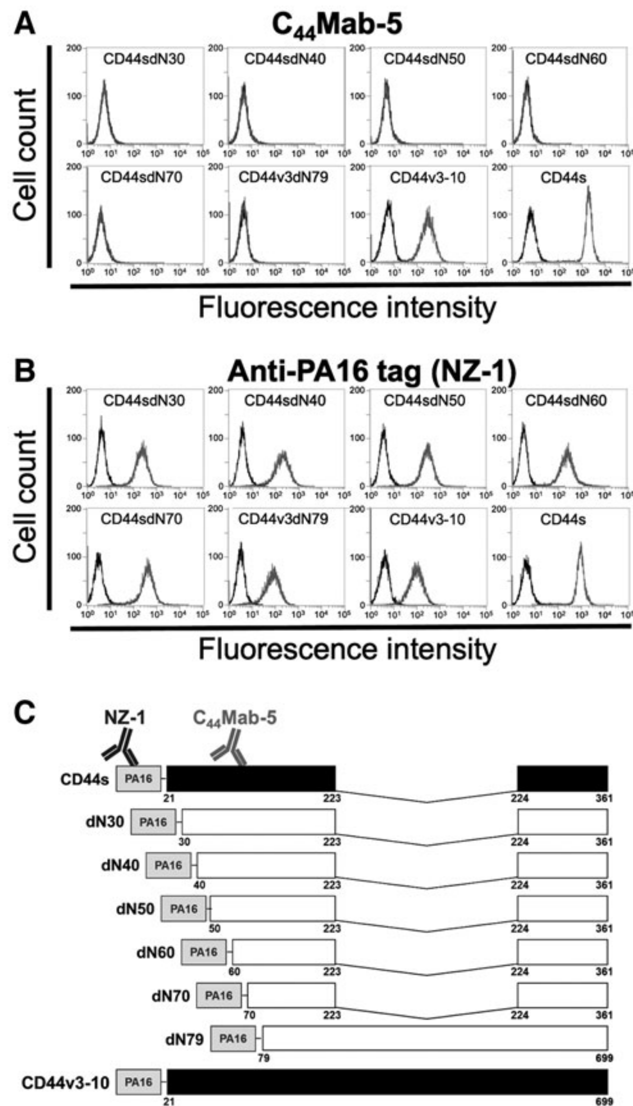

FIG. 1. Epitope mapping of C_{44}Mab-5 using deletion mutants of CD44s. **(A, B)** Deletion mutants of CD44s were analyzed using flow cytometry. Deletion mutants were expressed on CHO-K1 cells and then incubated with C_{44}Mab-5 **(A)**, or anti-PA16 tag antibody (NZ-1) **(B)** for 30 minutes at 4°C, followed by treatment with corresponding secondary antibodies. **(C)** Schematic illustration of epitope mapping of CD44s, CD44v3-10, and six deletion mutants of CD44s with PA16 tag at N-termini. Deletion mutants of CD44s: dN30, dN40, dN50, dN60, dN70, and dN79. Black bar: the positive reaction of C_{44}Mab-5. White bar: the negative reaction of C_{44}Mab-5. PA16: PA16 tag. CHO, Chinese hamster ovary.

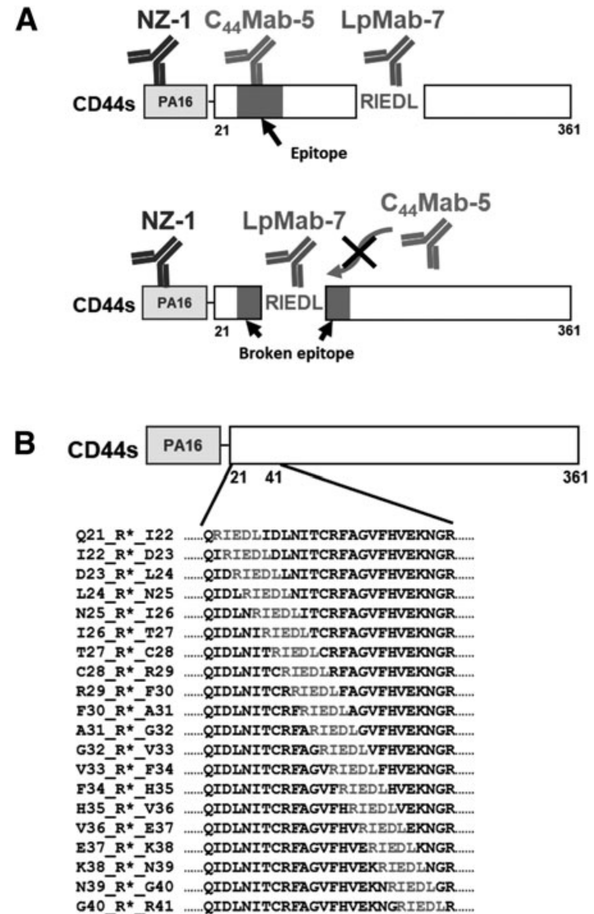

FIG. 2. Schematic illustration of REMAP method. RIEDL tag is inserted into CD44s. **(A)** C_{44}Mab-5 can bind to CD44s when RIEDL tag is inserted into any region, which is independent of the C_{44}Mab-5 epitope *(upper panel)*. In contrast, CD44s will not be detected by C_{44}Mab-5 when the conformation of the C_{44}Mab-5 epitope is disrupted by RIEDL insertion *(lower panel)*. **(B)** RIEDL tag was inserted into the expected epitope region within CD44s. REMAP, RIEDL insertion for epitope mapping.

positive control, NZ-1 against N-terminal PA16 tag detected 20 mutants (Fig. 3B). LpMab-7 detected most of the mutants, but did not detect E37_R*_K38, K38_R*_N39 (Fig. 3C), thereby suggesting that these AAs might be hidden inside the protein. Since RIEDL tag was not inserted in wild type CD44s, LpMab-7 did not react with CHO/CD44s (Fig. 3C). These results are summarized in Figure 4.

Discussion

To investigate the epitope of mAbs, alanine-scanning mutagenesis and peptide screening are often performed.[1,11,18–30] These methods are very useful for determining the linear epitope; however, we could not determine the conformational epitope by these methods. For investigating the epitope of C_{44}Mab-5 in this study, N-terminal deletion mutant analyses showed that the N-terminus of the epitope of C_{44}Mab-5 is located between Gln21 and Arg29 (Fig. 1). Next, we constructed the point mutants of CD44s that were substituted for alanine around the predicted epitope region. However, the result of flow cytometry analysis showed that all point mutants were detected by C_{44}Mab-5 (Supplementary Fig. S1); therefore, we could not determine the epitope by alanine-scanning mutagenesis. This is because the epitope of C_{44}Mab-5 will be a conformational epitope and substitution of one AA may not disrupt the conformation of the epitope region enough to inhibit the binding of C_{44}Mab-5. To determine the critical AAs of the epitope of C_{44}Mab-5, we

Downloaded by Yukinari Kato from www.liebertpub.com at 08/23/21. For personal use only.

Downloaded by Yukinari Kato from www.liebertpub.com at 08/23/21. For personal use only.

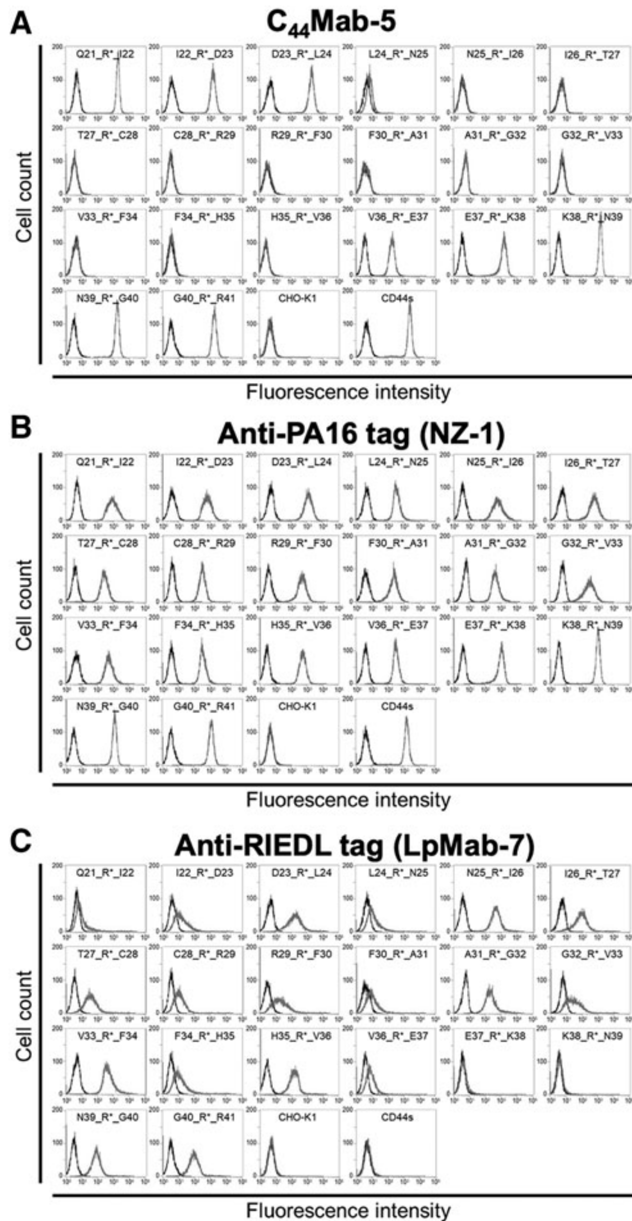

FIG. 3. Epitope mapping of C_{44}Mab-5 using RIEDL tag insertion mutants of CD44s. RIEDL tag was inserted into CD44s and RIEDL tag insertion mutants were analyzed using flow cytometry. RIEDL tag insertion mutants were expressed on CHO-K1 cells and incubated with **(A)** C_{44}Mab-5, **(B)** anti-PA16 tag antibody (NZ-1), or **(C)** anti-RIEDL tag antibody (LpMab-7) for 30 minutes at 4°C, followed by treatment with corresponding secondary antibodies.

employed REMAP method (Fig. 2A). Results of the flow cytometry analysis showed that some RIEDL tag insertion mutants lost their reactivity to C_{44}Mab-5 (Fig. 3A). This result suggests that insertion of RIEDL tag caused partial disruption of CD44s conformation and inhibited the binding of C_{44}Mab-5 to CD44s. Using REMAP method, we successfully determined that the critical epitope of C_{44}Mab-5 is located from Asn25 to Val36 (Fig. 4). These AAs are continuous, but may not produce a linear structure. They form a

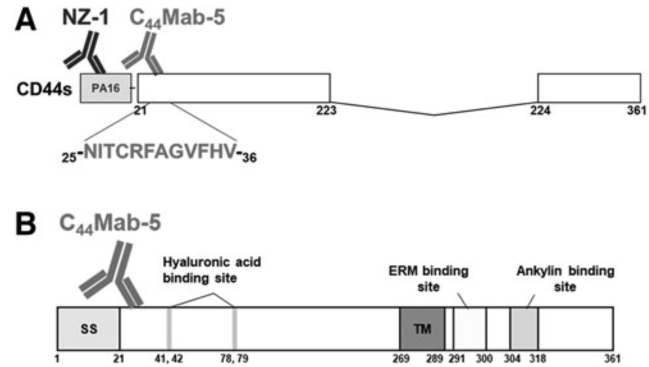

FIG. 4. Schematic illustration of epitope mapping of C_{44}Mab-5. **(A)** Schematic illustration of C_{44}Mab-5 and NZ-1. Asn25, Ile26, Thr27, Cys28, Arg29, Phe30, Ala31, Gly32, Val33, Phe34, His35, and Val36 are important for the binding of C_{44}Mab-5 to CD44s. **(B)** Schematic illustration of CD44s and epitope of C_{44}Mab-5. CD44s possesses hyaluronic acid-binding sites in the extracellular region and ERM binding site and ankyrin binding site in the intracellular region. C_{44}Mab-5 epitope is located near the N-terminus of CD44s. SS, signal sequence; TM, transmembrane domain.

beta sheet[31] and this conformation will be important for the binding of C_{44}Mab-5 to CD44s. The crystal structures of the extracellular hyaluronic acid (HA)-binding domain of human CD44 show that the binding epitope of C_{44}Mab-5 is located near the HA-binding site[31,32]; therefore, the binding of C_{44}Mab-5 to CD44s may affect the binding of HA to CD44s.

In this study, we showed that REMAP method is useful for the conformational epitope mapping of CD44s. RIEDL tag consists of only five AAs and is detected by anti-RIEDL tag mAb, LpMab-7. As shown in Figure 3C, most of the RIEDL tag insertion mutants were detected by LpMab-7; however, E37_R*_K38 and K38_R*_N39 were not detected. The peptide bonds between Glu37 and Lys38 and between Lys38 and Asn39 are not exposed on the surface of CD44s.[31] Since the inserted RIEDL tags might be buried in protein structure, RIEDL tags were not detected by LpMab-7 in those mutants. CD44 has been studied as a therapeutic target in several cancers and we recently developed 5-mG_{2a}-f, which showed ADCC and CDC activities against oral squamous cell carcinoma.[12] The epitope identification of C_{44}Mab-5 will be helpful for the development of antibody drug that targets CD44s in a future study.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This research was supported, in part, by Japan Agency for Medical Research and Development under Grant Nos. JP21am0401013 (to Y.K.) and JP21am0101078 (to Y.K.), and by the Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (KAKENHI) Grant Nos. 21K15523 (to T.A.), 21K07168 (to M.K.K.), and 19K07705 (to Y.K.).

Downloaded by Yukinari Kato from www.liebertpub.com at 08/23/21. For personal use only.

Supplementary Material

Supplementary Figure S1

References

1. Gershoni JM, Roitburd-Berman A, Siman-Tov DD, Tarnovitski Freund N, and Weiss Y: Epitope mapping: The first step in developing epitope-based vaccines. BioDrugs 2007;21:145–156.
2. Lo Conte L, Chothia C, and Janin J: The atomic structure of protein-protein recognition sites. J Mol Biol 1999;285: 2177–2198.
3. Geysen HM, Meloen RH, and Barteling SJ: Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. Proc Natl Acad Sci U S A 1984;81: 3998–4002.
4. Cunningham BC, and Wells JA: High-resolution epitope mapping of hGH-receptor interactions by alanine-scanning mutagenesis. Science 1989;244:1081–1085.
5. Ashkenazi A, Presta LG, Marsters SA, Camerato TR, Rosenthal KA, Fendly BM, and Capon DJ: Mapping the CD4 binding site for human immunodeficiency virus by alanine-scanning mutagenesis. Proc Natl Acad Sci U S A 1990;87: 7150–7154.
6. Bartolazzi A, Peach R, Aruffo A, and Stamenkovic I: Interaction between CD44 and hyaluronate is directly implicated in the regulation of tumor development. J Exp Med 1994;180:53–66.
7. Günthert U, Hofmann M, Rudy W, Reber S, Zöller M, Haussmann I, Matzku S, Wenzel A, Ponta H, and Herrlich P: A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell 1991;65:13–24.
8. Sleeman JP, Arming S, Moll JF, Hekele A, Rudy W, Sherman LS, Kreil G, Ponta H, and Herrlich P: Hyaluronate-independent metastatic behavior of CD44 variant-expressing pancreatic carcinoma cells. Cancer Res 1996;56:3134–3141.
9. Zahalka MA, Okon E, Gosslar U, Holzmann B, and Naor D: Lymph node (but not spleen) invasion by murine lymphoma is both CD44- and hyaluronate-dependent. J Immunol 1995;154:5345–5355.
10. Yan Y, Zuo X, and Wei D: Concise review: Emerging role of CD44 in cancer stem cells: A promising biomarker and therapeutic target. Stem Cells Transl Med 2015;4:1033–1043.
11. Furusawa Y, Yamada S, Itai S, Nakamura T, Fukui M, Harada H, Kaneko MK, and Kato Y: Elucidation of critical epitope of anti-rat podoplanin monoclonal antibody PMab-2. Monoclon Antib Immunodiagn Immunother 2018;37: 188–193.
12. Takei J, Kaneko MK, Ohishi T, Hosono H, Nakamura T, Yanaka M, Sano M, Asano T, Sayama Y, Kawada M, Harada H, and Kato Y: A defucosylated antiCD44 monoclonal antibody 5mG2af exerts antitumor effects in mouse xenograft models of oral squamous cell carcinoma. Oncol Rep 2020;44:1949–1960.
13. Asano T, Kaneko MK, and Kato Y: RIEDL tag: A novel pentapeptide tagging system for transmembrane protein purification. Biochem Biophys Rep 2020;23:100780.
14. Fujii Y, Kaneko M, Neyazaki M, Nogi T, Kato Y, and Takagi J: PA tag: A versatile protein tagging system using a super high affinity antibody against a dodecapeptide derived from human podoplanin. Protein Expr Purif 2014;95: 240–247.
15. Fujii Y, Matsunaga Y, Arimori T, Kitago Y, Ogasawara S, Kaneko MK, Kato Y, and Takagi J: Tailored placement of a turn-forming PA tag into the structured domain of a protein to probe its conformational state. J Cell Sci 2016;129: 1512–1522.
16. Kato Y, Kaneko MK, Kuno A, Uchiyama N, Amano K, Chiba Y, Hasegawa Y, Hirabayashi J, Narimatsu H, Mishima K, and Osawa M: Inhibition of tumor cell-induced platelet aggregation using a novel anti-podoplanin antibody reacting with its platelet-aggregation-stimulating domain. Biochem Biophys Res Commun 2006;349:1301–1307.
17. Yamada S, Itai S, Nakamura T, Yanaka M, Chang YW, Suzuki H, Kaneko MK, and Kato Y: Monoclonal antibody L(1)Mab-13 detected human PD-L1 in lung cancers. Monoclon Antib Immunodiagn Immunother 2018;37:110–115.
18. Chang YW, Kaneko MK, Yamada S, and Kato Y: Epitope mapping of monoclonal antibody PMab-52 against cat podoplanin. Monoclon Antib Immunodiagn Immunother 2018;37:95–99.
19. Chang YW, Yamada S, Kaneko MK, and Kato Y: Epitope mapping of monoclonal antibody PMab-38 against dog podoplanin. Monoclon Antib Immunodiagn Immunother 2017;36:291–295.
20. Kaneko MK, Furusawa Y, Sano M, Itai S, Takei J, Harada H, Fukui M, Yamada S, and Kato Y: Epitope mapping of the antihorse podoplanin monoclonal antibody PMab-202. Monoclon Antib Immunodiagn Immunother 2019;38:79–84.
21. Kaneko MK, Nakamura T, Kunita A, Fukayama M, Abe S, Nishioka Y, Yamada S, Yanaka M, Saidoh N, Yoshida K, Fujii Y, Ogasawara S, and Kato Y: ChLpMab-23: Cancer-specific human-mouse chimeric anti-podoplanin antibody exhibits antitumor activity via antibody-dependent cellular cytotoxicity. Monoclon Antib Immunodiagn Immunother 2017;36:104–112.
22. Kato Y, Takei J, Furusawa Y, Sayama Y, Sano M, Konnai S, Kobayashi A, Harada H, Takahashi M, Suzuki H, Yamada S, and Kaneko MK: Epitope mapping of anti-bear podoplanin monoclonal antibody PMab-247. Monoclon Antib Immunodiagn Immunother 2019;38:230–233.
23. Ogasawara S, Kaneko MK, Price JE, and Kato Y: Characterization of anti-podoplanin monoclonal antibodies: Critical epitopes for neutralizing the interaction between podoplanin and CLEC-2. Hybridoma 2008;27:259–267.
24. Sano M, Kaneko MK, and Kato Y: Epitope mapping of monoclonal antibody PMab-233 against Tasmanian devil podoplanin. Monoclon Antib Immunodiagn Immunother 2019;38:261–265.
25. Sayama Y, Sano M, Asano T, Furusawa Y, Takei J, Nakamura T, Yanaka M, Okamoto S, Handa S, Komatsu Y, Nakamura Y, Yanagawa M, Kaneko MK, and Kato Y: Epitope mapping of PMab-241, a lymphatic endothelial cell-specific anti-bear podoplanin monoclonal antibody. Monoclon Antib Immunodiagn Immunother 2020;39: 77–81.
26. Sayama Y, Sano M, Furusawa Y, Kaneko MK, and Kato Y: Epitope mapping of PMab-225 an anti-alpaca podoplanin monoclonal antibody using flow cytometry. Monoclon Antib Immunodiagn Immunother 2019;38:255–260.
27. Takei J, Itai S, Furusawa Y, Yamada S, Nakamura T, Sano M, Harada H, Fukui M, Kaneko MK, and Kato Y: Epitope mapping of anti-tiger podoplanin monoclonal antibody PMab-231. Monoclon Antib Immunodiagn Immunother 2019;38:129–132.

28. Yamada S, Itai S, Furusawa Y, Kaneko MK, and Kato Y: Epitope mapping of antipig podoplanin monoclonal antibody PMab-213. Monoclon Antib Immunodiagn Immunother 2019;38:224–229.
29. Yamada S, Itai S, Kaneko MK, Konnai S, and Kato Y: Epitope mapping of anti-mouse podoplanin monoclonal antibody PMab-1. Biochem Biophys Rep 2018;15: 52–56.
30. Yamada S, Kaneko MK, Itai S, Chang YW, Nakamura T, Yanaka M, Ogasawara S, Murata T, Uchida H, Tahara H, Harada H, and Kato Y: Epitope mapping of monoclonal antibody PMab-48 against dog podoplanin. Monoclon Antib Immunodiagn Immunother 2018;37:162–165.
31. Liu LK, and Finzel B: High-resolution crystal structures of alternate forms of the human CD44 hyaluronan-binding domain reveal a site for protein interaction. Acta Crystallogr F Struct Biol Commun 2014;70:1155–1161.
32. Bajorath J, Greenfield B, Munro SB, Day AJ, and Aruffo A: Identification of CD44 residues important for hyaluronan binding and delineation of the binding site. J Biol Chem 1998;273:338–343.

Address correspondence to:
Yukinari Kato
Department of Antibody Drug Development
Tohoku University Graduate School of Medicine
2-1, Seiryo-machi, Aoba-ku
Sendai, Miyagi 980-8575
Japan

E-mail: yukinarikato@med.tohoku.ac.jp

Received: May 14, 2021
Accepted: June 14, 2021

Downloaded by Yukinari Kato from www.liebertpub.com at 08/23/21. For personal use only.