MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 41, Number 1, 2022

DOI: 10.1089/mab.2021.0046

Epitope Mapping of an Anti-CD10 Monoclonal Antibody (MME/1870) Using Enzyme-Linked Immunosorbent Assay

Hiroki Kawabata,[1] Hiroyuki Suzuki,[2] Junko Takei,[1] Mika K. Kaneko,[1] and Yukinari Kato[1,2]

CD10 is a glycosylated transmembrane protein and is known as a membrane endopeptidase. CD10 is expressed on predifferentiated lymphocyte progenitor, epithelial, stromal, and tumor cells. Antibodies against CD10 are used for the diagnosis of follicular lymphoma. Anti-human CD10 monoclonal antibody (clone MME/1870) can be used for Western blotting and immunohistochemical analyses. This study examined the critical epitope of MME/1870 using enzyme-linked immunosorbent assay (ELISA) with synthesized peptides. First, we performed ELISA with deletion mutants, and MME/1870 reacted to the 501–520 amino acid sequence of CD10. Next, we analyzed the reaction to 20 point mutants, and MME/1870 did not recognize the alanine-substituted peptides of Y507A, I511A, I512A, and L515A. These results indicate that the binding epitope of MME/1870 includes Tyr507, Ile511, Ile512, and Leu515 of CD10.

Keywords: CD10, clone MME/1870, epitope mapping, monoclonal antibody, enzyme-linked immunosorbent assay

Introduction

CD10 is a type II transmembrane glycoprotein with a molecular weight of 100,000 and is known as a common acute lymphoblastic leukemia antigen.[1–3] CD10 was identified as membrane endopeptidase-24.11, an important role in the hydrolysis of biologically active peptides.[4] CD10 is expressed on predifferentiated lymphocyte progenitor cells, and the expression disappears when cells differentiate into T cells. In the case of B cell lineage, it disappears at the time of cell surface immunoglobulin expression. CD10 is detected on activated and proliferating B cells in the germinal center[5] and on bone marrow stromal cells and neutrophils.[6] CD10 is also expressed in human B cell malignancies, including follicular,[7] Burkitt, and lymphoblastic lymphomas.[8] Moreover, CD10 is expressed in normal epithelial cells of the small intestine, kidney, and lung, and its decreased or increased expression is observed in melanoma, thyroid cancer, and several carcinomas.[9]

CD10 is a neutral endopeptidase that cleaves at the amino side of hydrophobic residues of several hormones, including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin.[10,11] This function plays crucial roles in inactivating the physiological action of the peptides by lowering the extracellular concentration available for their receptors. Furthermore, CD10 cleaves growth factors, such as fibroblast growth factor 2, which induces Akt signaling in endothelial cells and stimulates angiogenesis.[12]

In addition to its enzymatic activity, CD10 regulates intracellular signaling pathways. For example, CD10 interacts with p85, a PI3K subunit, and Lyn kinase, which indirectly prevents Focal Adhesion Kinase (FAK) activation by PI3K.[13] Moreover, the association between CD10 and the tumor suppressor Phosphatase and Tensin Homolog Deleted from Chromosome 10 (PTEN) leads to decreased PIP3 phosphorylation, which activates the Akt pathway.[14]

Sensitive and specific monoclonal antibodies (mAbs) against CD10 are needed for the diagnosis of malignancies. Among them, anti-human CD10 mAb (clone MME/1870) is useful for Western blotting and immunohistochemical analyses but not for flow cytometry. The understanding of antibody–antigen binding by revealing the epitope is essential for future application to antibody technology. To clarify further characteristics of MME/1870, we performed epitope mapping using enzyme-linked immunosorbent assay (ELISA).

Departments of [1]Antibody Drug Development, and [2]Molecular Pharmacology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan.

Table 1. Identification of Anti-CD10 Monoclonal Antibody Epitope Using Synthesized Peptides by Enzyme-Linked Immunosorbent Assay

Peptides	*Sequence*	*Anti-CD10 mAb*
51–70	TYDDGISKSSDSIKSAARLI	–
61–80	DSIKSAARLIQNMDATTEPS	–
71–90	QNMDATTEPSTDFFKYASGG	–
81–100	TDFFKYASGGWLKRNVIPET	–
91–110	WLKRNVIPETSSRYGNFDIL	–
101–120	SSRYGNFDILRDELEVVLKD	–
111–130	RDELEVVLKDVLQEPKTEDI	–
121–140	VLQEPKTEDIVAVQKAKALY	–
131–150	VAVQKAKALYRSSINESAID	–
141–160	RSSINESAIDSRGGEPLLKL	–
151–170	SRGGEPLLKLLPDIYGWPVA	–
161–180	LPDIYGWPVATENWEQKYGA	–
171–190	TENWEQKYGASWTAEKAIAQ	–
181–200	SWTAEKAIAQLNSKYGKKVL	–
191–210	LNSKYGKKVLINLFVGTDDK	–
201–220	INLFVGTDDKNSVNHVIHID	–
211–230	NSVNHVIHIDQPRLGLPSRD	–
221–240	QPRLGLPSRDYYESTGIYKE	–
231–250	YYESTGIYKEASTAYVDFMI	–
241–260	ASTAYVDFMISVARLIRQEE	–
251–270	SVARLIRQEERLPIDENQLA	–
261–280	RLPIDENQLALEMNKVMELE	–
271–290	LEMNKVMELEKEIANATAKP	–
281–300	KEIANATAKPEDRNDPMLLY	–
291–310	EDRNDPMLLYNKMTLAQIQN	–
301–320	NKMTLAQIQNNFSLEINGKP	–
311–330	NFSLEINGKPFSWLNFTNEI	–
321–340	FSWLNFTNEIMSTVNISITN	–
331–350	MSTVNISITNEEDVVVYAPE	–
341–360	EEDVVVYAPEYLTKLKPILT	–
351–370	YLTKLKPILTKYSARDLQNL	–
361–380	KYSARDLQNLMSWRFIMDLV	–
371–390	MSWRFIMDLVSSLSRTYKES	–
381–400	SSLSRTYKESRNAFRKALYG	–
391–410	RNAFRKALYGTTSETATWRR	–
401–420	TTSETATWRRSANYVNGNME	–
411–430	SANYVNGNMENAVGRLYVEA	–
421–440	NAVGRLYVEAAFAGESKHVV	–
431–450	AFAGESKHVVEDLIAQIREV	–
441–460	EDLIAQIREVFIQTLDDLTW	–
451–470	FIQTLDDLTWMDAETKKRAE	–
461–480	MDAETKKRAEEKALAIKERI	–
471–490	EKALAIKERIGYPDDIVSND	–
481–500	GYPDDIVSNDNKLNNEYLEL	–
491–510	NKLNNEYLELNYKEDEYFEN	–
501–520	NYKEDEYFENIIQNLKFSQS	+++
511–530	IIQNLKFSQSKQLKKLREKV	–
521–540	KQLKKLREKVDKDEWISGAA	–
531–550	DKDEWISGAAVVNAFYSSGR	–
541–560	VVNAFYSSGRNQIVFPAGIL	–
551–570	NQIVFPAGILQPPFFSAQQS	–
561–580	QPPFFSAQQSNSLNYGGIGM	–
571–590	NSLNYGGIGMVIGHEITHGF	–
581–600	VIGHEITHGFDDNGRNFNKD	–
591–610	DDNGRNFNKDGDLVDWWTQQ	–
601–620	GDLVDWWTQQSASNFKEQSQ	–
611–630	SASNFKEQSQSMVYQYGNFS	–
621–640	SMVYQYGNFSWDLAGGQHLN	–
631–650	WDLAGGQHLNGINTLGENIA	–
641–660	GINTLGENIADNGGLGQAYR	–
651–670	DNGGLGQAYRAYQNYIKKNG	–
661–680	AYQNYIKKNGEEKLLPGLDL	–
671–690	EEKLLPGLDLNHKQLFFLNF	–
681–700	NHKQLFFLNFAQVWSGTYRP	–
691–710	AQVWSGTYRPEYAVNSIKTD	–
701–720	EYAVNSIKTDVHSPGNFRII	–
711–730	VHSPGNFRIIGTLQNSAEFS	–
721–740	GTLQNSAEFSEAFHSRKNSY	–
731–750	EAFHSRKNSYMNPEKKSRVW	–

(*continued*)

Table 1. (Continued)

+++, OD655 ≧0.5; ++, 0.3 ≦OD655 < 0.5; +, 0.1 ≦OD655 < 0.3; –, OD655 < 0.1.
mAb, monoclonal antibody.

Materials and Methods

Antibodies

Anti-CD10 mAb, clone MME/1870 (cat. no. ab238021), was purchased from Abcam (Cambridge, UK).

CD10 peptides

CD10 peptides (accession no. NM_000902.3), including 69 deletion mutants (Table 1) and 20 point mutants (Table 2), were synthesized by utilizing PEPScreen (Sigma-Aldrich Corp., St. Louis, MO).

Enzyme-linked immunosorbent assay

Synthesized CD10 peptides (Tables 1 and 2) were immobilized on Nunc Maxisorp 96-well immunoplates (Thermo Fisher Scientific Inc., Waltham, MA) at a concentration of 10 μg/mL for 30 minutes at 37°C. After washing with

Table 2. Identification of Anti-CD10 Monoclonal Antibody Epitope Using Alanine-Substituted Peptides by Enzyme-Linked Immunosorbent Assay

Peptides	*Sequence*	*Anti-CD10 mAb*
N501A	AYKEDEYFENIIQNLKFSQS	+++
Y502A	NAKEDEYFENIIQNLKFSQS	+++
K503A	NYAEDEYFENIIQNLKFSQS	+++
E504A	NYKADEYFENIIQNLKFSQS	++
D505A	NYKEAEYFENIIQNLKFSQS	+
E506A	NYKEDAYFENIIQNLKFSQS	+++
Y507A	NYKEDEAFENIIQNLKFSQS	–
F508A	NYKEDEYAENIIQNLKFSQS	+
E509A	NYKEDEYFANIIQNLKFSQS	+++
N510A	NYKEDEYFEAIIQNLKFSQS	+++
I511A	NYKEDEYFENAIQNLKFSQS	–
I512A	NYKEDEYFENIAQNLKFSQS	–
Q513A	NYKEDEYFENIIANLKFSQS	+++
N514A	NYKEDEYFENIIQALKFSQS	+
L515A	NYKEDEYFENIIQNAKFSQS	–
K516A	NYKEDEYFENIIQNLAFSQS	+++
F517A	NYKEDEYFENIIQNLKASQS	+++
S518A	NYKEDEYFENIIQNLKFAQS	+++
Q519A	NYKEDEYFENIIQNLKFSAS	+++
S520A	NYKEDEYFENIIQNLKFSQA	+++

+++, OD655 ≧0.5; ++, 0.3 ≦OD655 < 0.5; +, 0.1 ≦OD655 < 0.3; –, OD655 < 0.1.

phosphate-buffered saline containing 0.05% Tween20 (PBST; Nacalai Tesque, Inc., Kyoto, Japan), wells were blocked with 1% bovine serum albumin-containing PBST for 30 min at 37°C. The plates were incubated with 1 μg/m of MME/1870, followed by a peroxidase-conjugated anti-mouse immunoglobulins (1:2000 diluted; Agilent Technologies Inc., Santa Clara, CA). Enzymatic reactions were performed using the ELISA POD Substrate TMB Kit (Nacalai Tesque, Inc.). Optical density was measured at 655 nm using an iMark microplate reader (Bio-Rad Laboratories, Inc., Berkeley, CA).

Results

Epitope mapping of an anti-CD10 mAb using deletion mutants

To determine the binding epitope of MME/1870, we synthesized 69 CD10 peptides with their length of 20 amino acids (aa). The peptides are summarized in Table 1. The results showed that MME/1870 reacted to the 501–520 aa sequence ($_{501}$-NYKEDEYFENIIQNLKFSQS-$_{520}$) of CD10. We summarized the results in Figure 1.

Epitope mapping of an anti-CD10 mAb using point mutants

Then, we synthesized 20 alanine-substituted peptides of 501–520 aa of CD10 (Table 2). MME/1870 exhibited reaction to N501A, Y502A, K503A, E504A, D505A, E506A, F508A, E509A, N510A, Q513A, N514A, K516A, F517A, S518A, Q519A, S520A, and wild-type 501–520 aa, whereas it showed no reaction to Y507A, I511A, I512A, and L515A (Fig. 2A). The results indicate that Tyr507, Ile511, Ile512, and Leu515 are the critical binding epitope of MME/1870. We summarized the results in Figure 2B.

Discussion

In this study, we investigated the binding epitope of MME/1870 using synthesized peptides and determined Tyr507, Ile511, Ile512, and Leu515 as minimum epitope (Fig. 2B). We could not rule out the possibility that Glu504, Asp505, Phe508, and Asn514 are involved in the recognition since the reactivities were reduced to a lesser extent (Fig. 2A). Peptide scanning is a simple and useful technique for determining a linear epitope. However, the peptide structure is unfolded and different from native proteins. According to the X-ray crystal structure analysis, the epitope region forms a helical structure.[15] In a previous study, we developed two novel epitope mapping methods, such as the RIEDL insertion for epitope mapping (REMAP) method to insert the RIEDL tag, and His-tag insertion for epitope mapping (HisMAP) method to insert five His tags into targeted proteins.

These methods are useful for determining both linear and conformational epitopes. We have already determined the epitope of anti-EGFR mAbs (EMab-51 and EMab-134),[16,17] an anti-CD44 mAb (C_{44}Mab-46),[18] and an anti-CD20 mAb

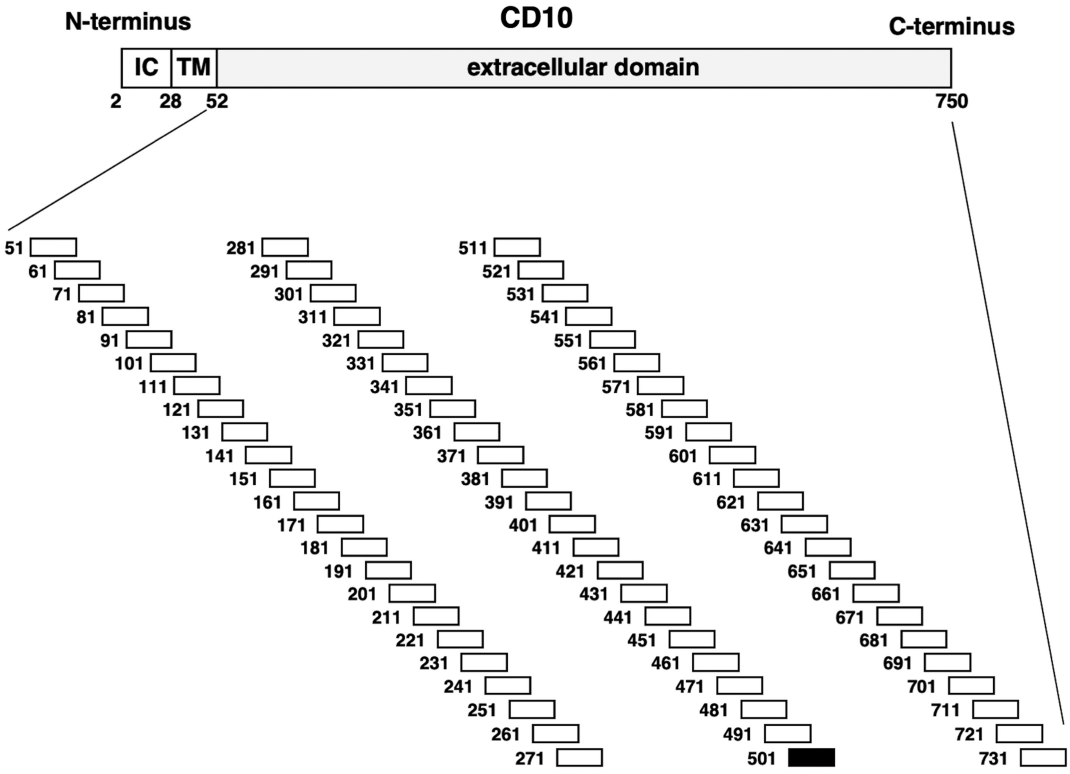

FIG. 1. Determination of the anti-CD10 mAb (clone MME/1870) epitope by ELISA using deletion mutants. Schematic illustration of CD10 and synthesized peptides. Synthesized peptides of CD10 were immobilized on immunoplates. The plates were incubated with anti-CD10 mAb (1 μg/mL), followed by incubation with peroxidase-conjugated anti-mouse immunoglobulins. The peptides sequence and the result of ELISA are summarized in Table 1. A *black bar* (501–520 aa) indicates the anti-CD10 mAb epitope. aa, amino acids; ELISA, enzyme-linked immunosorbent assay; IC, intracellular domain; mAb, monoclonal antibody; TM, transmembrane domain.

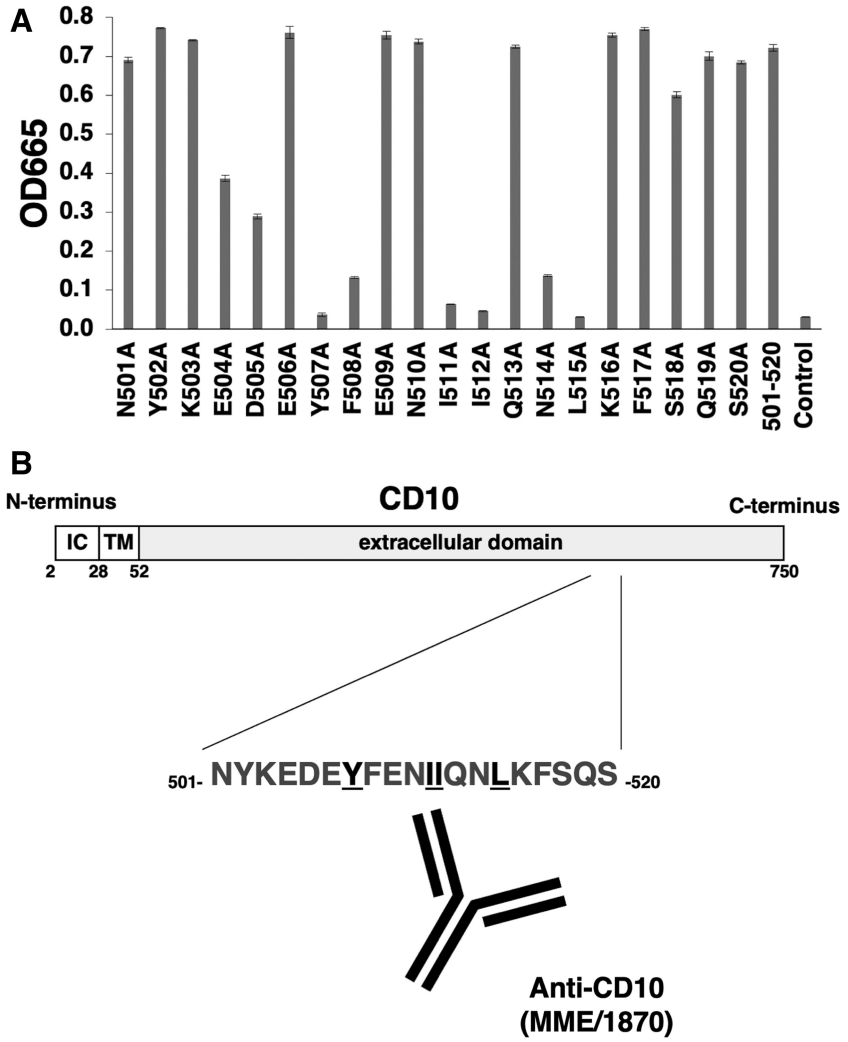

FIG. 2. Determination of the anti-CD10 mAb (clone MME/1870) epitope by ELISA using point mutants. **(A)** Synthesized peptides of CD10 were immobilized on immunoplates. The plates were incubated with anti-CD10 mAb (1 μg/mL), followed by peroxidase-conjugated anti-mouse immunoglobulins. **(B)** Schematic illustration of CD10 and the anti-CD10 mAb epitope. The anti-CD10 mAb epitope of CD10 involves Tyr507, Ile511, Ile512, and Leu515.

(C_{20}Mab-60).[19] Application of these strategies will be important for further understanding of the epitope of MME/1870. Furthermore, this epitope is not conserved among the family member of mammalian neutral endopeptidases, including endothelin-converting enzymes (ECE1/2), Kell metalloendopeptidase, and phosphate-regulating endopeptidase homolog X-linked. Therefore, immunization of the epitope is thought to be important for the further development of mAbs.

CD10 belongs to the M13 family of mammalian neutral endopeptidases and has an N-terminal cytoplasmic domain, followed by a single transmembrane domain and C-terminal extracellular domain that contains the active site.[6,10,11] The X-ray crystal structure of the extracellular domain (52–749 aa) of CD10 was solved with a metalloproteinase inhibitor phosphoramidon.[15] The structure reveals two consensus sequences HExxH and ExxxD, multiplying connected folding domains that embrace a large central cavity containing the active site. The inhibitor is bound to this cavity, which provides a detailed ligand recognition. Furthermore, $_{543}$-NAFY-$_{546}$, closely localized to the identified epitope, forms a β-strand, which also participates in ligand recognition. There is no information on whether MME/1870 affects the enzymatic activity of CD10. Further studies are warranted for the inhibitory effect of MME/1870 on CD10-mediated ligand recognition and endopeptidase activity.

Recently, the functional anti-CD10 antibody (JMAM-1) has been reported.[20] JMAM-1 has a cytostatic effect on mesothelioma MSTO-211H cells *in vitro* and exhibits prolonged survival of NCI-H226 tumor-bearing mice. These results expect the use of anti-CD10 antibody for pathological diagnosis and tumor therapy. However, the detailed mechanism of the antitumor effect has not been investigated. Therefore, in cancer treatment, the functional elucidation of anti-CD10 mAbs, including JMAM-1 and MME/1870, is required for future application.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This research was supported in part by Japan Agency for Medical Research and Development (AMED) under grant nos.

JP21am0401013 (to Y.K.) and JP21am0101078 (to Y.K.), and by the Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (KAKENHI) grant nos. 21K07168 (to M.K.K.) and 19K07705 (to Y.K.).

References

1. Billing R, Minowada J, Cline M, Clark B, and Lee K: Acute lymphocytic leukemia-associated cell membrane antigen. J Natl Cancer Inst 1978;61:423–429.
2. Borella L, Sen L, and Casper JT: Acute lymphoblastic leukemia (ALL) antigens detected with antisera to E rosette-froming and non-E rosette-forming ALL blasts. J Immunol 1977;118:309–315.
3. Minowada J, Janossy G, Greaves MF, Tsubota T, Srivastava BI, Morikawa S, and Tatsumi E: Expression of an antigen associated with acute lymphoblastic leukemia in human leukemia-lymphoma cell lines. J Natl Cancer Inst 1978;60:1269–1277.
4. Kenny AJ, O'Hare MJ, and Gusterson BA: Cell-surface peptidases as modulators of growth and differentiation. Lancet 1989;2:785–787.
5. Béné MC: Immunophenotyping of acute leukaemias. Immunol Lett 2005;98:9–21.
6. Erdös EG, and Skidgel RA: Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. Faseb J 1989;3:145–151.
7. Freedman A: Follicular lymphoma: 2018 update on diagnosis and management. Am J Hematol 2018;93:296–305.
8. Cortelazzo S, Ponzoni M, Ferreri AJ, and Hoelzer D: Lymphoblastic lymphoma. Crit Rev Oncol Hematol 2011; 79:330–343.
9. Mishra D, Singh S, and Narayan G: Role of B cell development marker CD10 in cancer progression and prognosis. Mol Biol Int 2016;2016:4328697.
10. Roques BP, Noble F, Daugé V, Fournié-Zaluski MC, and Beaumont A: Neutral endopeptidase 24.11: Structure, inhibition, and experimental and clinical pharmacology. Pharmacol Rev 1993;45:87–146.
11. Turner AJ, and Tanzawa K: Mammalian membrane metallopeptidases: NEP, ECE, KELL, and PEX. Faseb J 1997; 11:355–364.
12. Goodman OB, Jr., Febbraio M, Simantov R, Zheng R, Shen R, Silverstein RL, and Nanus DM: Neprilysin inhibits angiogenesis via proteolysis of fibroblast growth factor-2. J Biol Chem 2006;281:33597–33605.
13. Maguer-Satta V, Besançon R, and Bachelard-Cascales E: Concise review: Neutral endopeptidase (CD10): A multifaceted environment actor in stem cells, physiological mechanisms, and cancer. Stem Cells 2011;29:389–396.
14. Sumitomo M, Shen R, and Nanus DM: Involvement of neutral endopeptidase in neoplastic progression. Biochim Biophys Acta 2005;1751:52–59.
15. Oefner C, D'Arcy A, Hennig M, and Winkler FK, Dale GE: Structure of human neutral endopeptidase (Neprilysin) complexed with phosphoramidon. J Mol Biol 2000;296: 341–349.
16. Sano M, Kaneko MK, Aasano T, and Kato Y: Epitope mapping of an antihuman EGFR monoclonal antibody (EMab-134) using the REMAP method. Monoclon Antib Immunodiagn Immunother 2021;40:191–195.
17. Nanamiya R, Sano M, Asano T, Yanaka M, Nakamura T, Saito M, Tanaka T, Hosono H, Tateyama N, Kaneko MK, and Kato Y: Epitope mapping of an anti-human epidermal growth factor receptor monoclonal antibody (EMab-51) using the RIEDL insertion for epitope mapping method. Monoclon Antib Immunodiagn Immunother 2021;40:149–155.
18. Asano T, Kaneko MK, Takei J, Tateyama N, and Kato Y: Epitope mapping of the anti-CD44 monoclonal antibody (C44Mab-46) using the REMAP method. Monoclon Antib Immunodiagn Immunother 2021;40:156–161.
19. Asano T, Takei J, Furusawa Y, Saito M, Suzuki H, Kaneko MK, and Y. K: Epitope mapping of an anti-CD20 monoclonal antibody (C20Mab-60) using the HisMAP method. Monoclon Antib Immunodiagn Immunother 2021;40:243–249.
20. Mizutani N, Abe M, Kajino K, and Matsuoka S: A new CD10 antibody inhibits the growth of malignant mesothelioma. Monoclon Antib Immunodiagn Immunother 2021;40:21–27.

Address correspondence to:
Yukinari Kato
Department of Molecular Pharmacology
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: September 17, 2021
Accepted: January 11, 2021