MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 41, Number 3, 2022

DOI: 10.1089/mab.2022.0013

TgMab-2: An Anti-human T Cell Immunoglobulin and Immunoreceptor Tyrosine-Based Inhibitory Motif Domain Monoclonal Antibody for Immunocytochemistry

Masaki Saito,[1] Hiroyuki Suzuki,[1] Mika K. Kaneko,[2] and Yukinari Kato[1,2]

T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) is one of the immune checkpoint molecules. TIGIT is expressed in T or natural killer (NK) cells and is upregulated in several cancers. Because TIGIT suppresses the antitumor activity of the T or NK cells by binding to its ligand, such as CD155, CD112, and CD113, TIGIT can be a molecular marker or a therapeutic target for cancer immunotherapy. We previously developed an anti-human TIGIT (hTIGIT) monoclonal antibody (mAb; clone TgMab-2; mouse IgG_1, kappa) by the Cell-Based Immunization and Screening method. TgMab-2 binds to hTIGIT with high binding affinity in flow cytometry. In this study, we investigated the availability of TgMab-2 and its recombinant mAb (recTgMab-2) in immunocytochemistry. We found that TgMab-2 and recTgMab-2 bind to hTIGIT-overexpressed Chinese hamster ovary (CHO)-K1 cells, but not parental CHO-K1 cells, indicating that both mAbs specifically recognize hTIGIT. Furthermore, both mAbs recognized endogenous hTIGIT expressed in human NK cells in immunocytochemistry. These results demonstrate that TgMab-2 and recTgMab-2 are applicable for immunocytochemistry against hTIGIT.

Keywords: TIGIT, TgMab-2, monoclonal antibody, immunocytochemistry

Introduction

$CD4^+$ T, $CD8^+$ T, and natural killer (NK) cells suppress tumor progression by antitumor immunity.[1] Tumor cells, in turn, escape from the immune cell recognition by activating immune checkpoint molecules, including cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) and by suppressing the functions of T or NK cells. Specific monoclonal antibodies (mAbs) against CTLA-4, PD-1, and PD-1 ligand 1 (PD-L1) could block their function, and have provided great benefits in improving the prognosis of cancer patients.[2] However, due to the limited number of patients who respond to those mAbs, the development of novel mAbs against other immune checkpoint molecules has been desired.[3]

T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) is another immune checkpoint molecule, which is expressed in $CD4^+$ T, $CD8^+$ T, NK, and regulatory T (Treg) cells.[4,5] TIGIT is a type 1 transmembrane protein, which possesses an extracellular immunoglobulin domain and an intracellular immunoreceptor tyrosine-based inhibitory motif domain.[4] CD155 (poliovirus receptor [PVR]), CD112 (PVR-like protein 2), and CD113 (PVR-like protein 3) are identified as TIGIT ligands that are expressed in antigen-presenting cells and cancer cells.[6]

TIGIT interacts with CD155, CD112, and CD113 with high, moderate, and low affinities, respectively.[4,5] The binding of CD155 to TIGIT suppresses the activity of NK cells through the phosphorylation of Y225 on the immunoreceptor tail tyrosine-like motif of TIGIT, the recruitment of a cytosolic adaptor protein Grb2 and SH2 domain-containing tyrosine phosphatase-1, and the subsequent termination of phosphatidylinositol 3-kinase and mitogen-activated protein kinase signaling.[7]

Elevated expression of TIGIT and its ligands is identified in tumor-infiltrating lymphocytes.[6] For example, TIGIT is increased in Tregs of melanoma patients[8] and $CD8^+$ T cells of gastric cancer patients.[9] TIGIT is also increased in

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

tumor-infiltrating NK cells or $CD8^+$ T cells in mouse xenograft models of colorectal cancer, breast cancer, melanoma, and fibrosarcoma.[10] In addition, CD155 is upregulated in melanoma, colorectal, gastric, and pancreatic carcinomas,[9,11–13] whereas CD112 is upregulated in breast, gastric, and ovarian carcinomas.[9,14]

These reports suggest that TIGIT can be a novel molecular marker or a target molecule for cancer immunotherapy. Moreover, despite advances in research of physiological functions of TIGIT, the regulatory mechanisms of TIGIT in the TIGIT-overexpressing $CD8^+$ T and NK cells have remained unclear. Thus, the development of novel mAbs against TIGIT has been required.

We have developed mAbs against membrane proteins by the Cell-Based Immunization and Screening (CBIS) method, including C–C motif chemokine receptor 3 (CCR3),[15,16] CCR8,[17–19] CCR9,[20] CD10,[21,22] CD19,[23] CD20,[24,25] CD44,[26] CD133,[27] EpCAM,[28,29] HER3,[30] KLRG1,[15] PD-L1,[31] podoplanin,[32–46] and TROP2.[47,48] We have also established an anti-human TIGIT (hTIGIT) mAb (clone TgMab-2; mouse IgG_1, kappa).[49] TgMab-2 reacts to hTIGIT with high binding affinity in flow cytometry.[49] In this study, we investigated whether TgMab-2 and its recombinant mAb (recTgMab-2) could recognize endogenous and exogenous hTIGIT in immunocytochemistry.

Materials and Methods

Cell lines

Chinese hamster ovary (CHO)-K1 was obtained from the American Type Culture Collection (Manassas, VA). hTIGIT-overexpressed CHO-K1 (CHO/hTIGIT) was established in our previous report.[49] CHO-K1 and CHO/hTIGIT were cultured in Roswell Park Memorial Institute 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan), supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc., Waltham, MA), 0.25 μg/mL of amphotericin B, 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Nacalai Tesque, Inc.). The cells were maintained in a humidified atmosphere under 5% CO_2 and 95% air condition at 37°C. Human NK cells (donor lot. 4022602, purity >70%) were purchased from Takara Bio (Shiga, Japan).

Antibodies

TgMab-2 was developed in our previous report.[49] Recombinant TgMab-2 (recTgMab-2) was generated by subcloning V_H and C_H of complementary DNAs (cDNAs) of TgMab-2 into the pCAG-Neo vector, along with V_L and C_L cDNAs of TgMab-2 into the pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), respectively. An anti-hTIGIT mAb (clone A15153G) was purchased from BioLegend (San Diego, CA). Alexa Fluor 488-conjugated anti-mouse IgG was purchased from Cell Signaling Technology, Inc. (Danvers, MA).

Immunocytochemistry of adherent cells

CHO-K1 and CHO/hTIGIT were seeded on acid-wash coverslips. They were fixed with 4% paraformaldehyde (PFA) contained in phosphate-buffered saline (PBS) for 10 min and quenched with 50 mM NH_4Cl contained in PBS supplemented with 0.2 mM Ca^{2+} and 2 mM Mg^{2+} (PBSc/m) for 10 min. Then, the cells were blocked in BPA buffer (PBSc/m supplemented with 0.5% bovine serum albumin and 0.02% sodium azide) for 30 min, and incubated with primary antibodies (10 μg/mL in BPA buffer) for 1 h and Alexa Fluor 488-conjugated anti-mouse IgG (1:400 dilution in BPA buffer) for 45 min.

Finally, the cells were mounted using ProLong Glass antifade mounting medium (Thermo Fisher Scientific, Inc.). The cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific, Inc.). Fluorescence images were acquired on a BZ-X800 digital microscope (Keyence, Osaka, Japan) with a 40× objective.

Immunocytochemistry of suspension cells

The suspension of NK cells was centrifuged at 270× *g* for 5 min. The obtained cell pellet was suspended in 4% PFA in PBS for 10 min, followed by 50 mM NH_4Cl in PBSc/m for 10 min. After centrifugation, NK cells were suspended in BPA buffer for 30 min, primary antibodies (10 μg/mL in BPA buffer) for 2 h, and Alexa Fluor 488-conjugated anti-mouse IgG (1:400 dilution BPA buffer) for 45 min. Subsequently, NK cells were suspended in ProLong Glass antifade mounting medium and mounted on a slide glass. The cell nuclei were stained with DAPI. Fluorescence images were acquired on a BZ-X800 digital microscope with a 40× objective.

Results

We previously showed that TgMab-2 specifically recognizes CHO/hTIGIT cells in flow cytometry.[49] In this study, we investigated the availability of TgMab-2 and recTgMab-2 in immunocytochemistry. We found TgMab-2 and recTgMab-2, but not buffer control, reacted to CHO/hTIGIT cells (Fig. 1A). In contrast, TgMab-2 and recTgMab-2 did not bind to parental CHO-K1 cells (Fig. 1B). Another anti-hTIGIT mAb (A15153G) also reacted to CHO/hTIGIT cells, but not to CHO-K1 cells (Fig. 1A, B). This result demonstrates that TgMab-2 and recTgMab-2 specifically recognize exogenous hTIGIT in immunocytochemistry.

Next, we have applied TgMab-2 and recTgMab-2 in NK cells to investigate whether both antibodies recognize endogenously expressing hTIGIT. We found that TgMab-2 and recTgMab-2, as well as A15153G, showed sensitive fluorescent signals in NK cells (Fig. 1C), indicating that TgMab-2 and recTgMab-2 reacted to endogenous hTIGIT in immunocytochemistry.

Discussion

This study demonstrated that TgMab-2 and recTgMab-2 specifically recognize endogenous and exogenous hTIGIT in immunocytochemistry. These mAbs would be powerful tools for the diagnosis of hTIGIT-positive cancers through detection of the tumor-infiltrating NK and $CD8^+$ T cells.

Importantly, TgMab-2 and recTgMab-2 provided high-contrast fluorescent images against both exogenously and endogenously expressing hTIGIT in CHO/hTIGIT and NK cells, respectively. We speculate that mAbs, developed by the CBIS method,[27] are suitable in immunocytochemistry because a mammalian cell line, which stably expresses a target

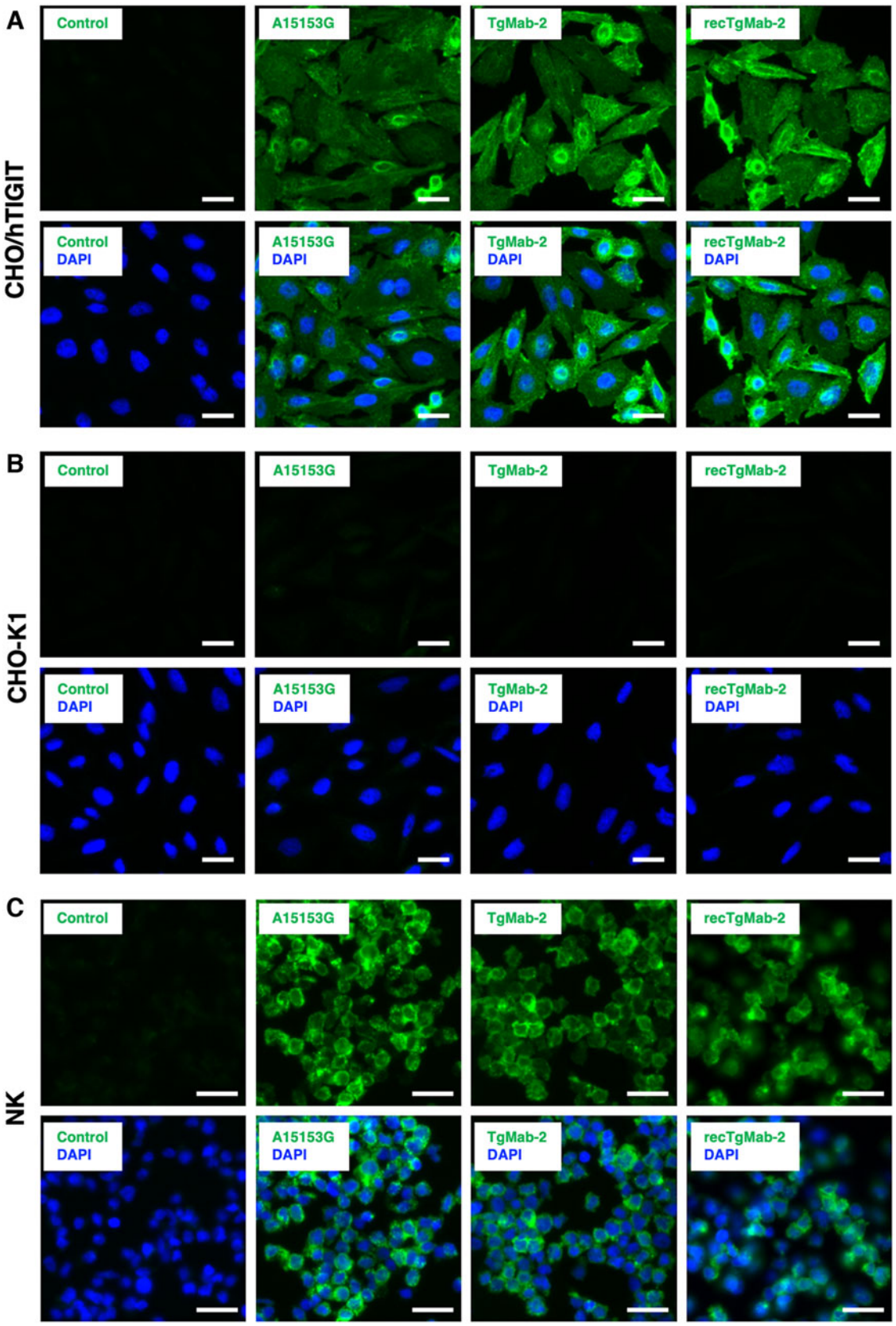

FIG. 1. Immunocytochemistry using TgMab-2 and recTgMab-2. **(A, B)** CHO/hTIGIT cells **(A)** or CHO-K1 **(B)** cells were treated with buffer control, 10 μg/mL of A15153G, 10 μg/mL of TgMab-2, or 10 μg/mL of recTgMab-2 for 1 h. The cells were further treated with Alexa 488-conjugated anti-mouse IgG and DAPI for 45 min. **(C)** Immunocytochemistry of NK cells using TgMag-2 and recTgMab-2. NK cells were treated with buffer control, 10 μg/mL of A15153G, 10 μg/mL of TgMab-2, or 10 μg/mL of recTgMab-2 for 2 h. The cells were further treated with Alexa 488-conjugated anti-mouse IgG and DAPI for 45 min. Scale bars; 20 μm. CHO, Chinese hamster ovary; DAPI, 4',6-diamidino-2-phenylindole; hTIGIT, human TIGIT; IgG, immunoglobulin G; NK, natural killer; TIGIT, T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain.

membrane protein, is used for an immunogen. Furthermore, the conformation and post-translational modification of the immunogen would be physiological.

In fact, we have confirmed that an anti-CCR3 mAb [clone C_3Mab-2[50]], anti-CCR8 mAbs [clone C_8Mab-1,[19] C_8Mab-2,[51] and C_8Mab-3[18]], and an anti-CCR9 mAb [clone C_9Mab-1[52]] also provided high-contrast images against both endogenously and exogenously expressing target molecules in immunocytochemistry.

We consider that the high-contrast images by TgMab-2 and recTgMab-2 would enable us to identify the cellular distribution of hTIGIT and support elucidate the physiological functions of hTIGIT. To further clarify the function of TIGIT using TgMab-2 and recTgMab-2, we need to investigate whether both mAbs are applicable for immunohistochemistry, immunoprecipitation, and Western blotting in the future study.

Some studies have revealed that blockade of TIGIT by its specific mAbs elicited antitumor responses and tumor regression, including colorectal carcinoma, breast cancer, melanoma, and fibrosarcoma.[10,53,54] Interestingly, co-blockade of TIGIT with PD-1 and CTLA-4 more potently elicits antitumor responses.[53–55] Moreover, co-blockade of TIGIT and PD-1 together with CD40 agonist suppressed the progression of pancreatic cancer.[56] In the future, it is necessary to investigate the antitumor responses of TgMab-2 and recTgMab-2, in addition to the antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity activities.

Author Disclosure Statement

The authors have no conflict of interest.

Funding Information

This research was supported, in part, by Japan Agency for Medical Research and Development (AMED) under Grant Numbers: JP22ama121008 (to Y.K.), JP22am0401013 (to Y.K.), JP22bm1004001 (to Y.K.), JP22ck0106730 (to Y.K.), and JP21am0101078 (to Y.K.).

References

1. Huntington ND, Cursons J, and Rautela J: The cancer-natural killer cell immunity cycle. Nat Rev Cancer 2020; 20:437–454.
2. Ribas A, and Wolchok JD: Cancer immunotherapy using checkpoint blockade. Science 2018;359:1350–1355.
3. Kraehenbuehl L, Weng CH, Eghbali S, Wolchok JD, and Merghoub T: Enhancing immunotherapy in cancer by targeting emerging immunomodulatory pathways. Nat Rev Clin Oncol 2022;19:37–50.
4. Yu X, Harden K, Gonzalez LC, Francesco M, Chiang E, Irving B, Tom I, Ivelja S, Refino CJ, Clark H, Eaton D, and Grogan JL: The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol 2009;10:48–57.
5. Stanietsky N, Simic H, Arapovic J, Toporik A, Levy O, Novik A, Levine Z, Beiman M, Dassa L, Achdout H, Stern-Ginossar N, Tsukerman P, Jonjic S, and Mandelboim O: The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. Proc Natl Acad Sci U S A 2009;106:17858–17863.
6. Harjunpaa H, and Guillerey C: TIGIT as an emerging immune checkpoint. Clin Exp Immunol 2020;200:108–119.
7. Liu S, Zhang H, Li M, Hu D, Li C, Ge B, Jin B, and Fan Z: Recruitment of Grb2 and SHIP1 by the ITT-like motif of TIGIT suppresses granule polarization and cytotoxicity of NK cells. Cell Death Differ 2013;20:456–464.
8. Fourcade J, Sun Z, Chauvin JM, Ka M, Davar D, Pagliano O, Wang H, Saada S, Menna C, Amin R, Sander C, Kirkwood JM, Korman AJ, and Zarour HM: CD226 opposes TIGIT to disrupt Tregs in melanoma. JCI Insight 2018;3:e121157.
9. Xu D, Zhao E, Zhu C, Zhao W, Wang C, Zhang Z, and Zhao G: TIGIT and PD-1 may serve as potential prognostic biomarkers for gastric cancer. Immunobiology 2020;225: 151915.
10. Zhang Q, Bi J, Zheng X, Chen Y, Wang H, Wu W, Wang Z, Wu Q, Peng H, Wei H, Sun R, and Tian Z: Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. Nat Immunol 2018; 19:723–732.
11. Masson D, Jarry A, Baury B, Blanchardie P, Laboisse C, Lustenberger P, and Denis MG: Overexpression of the CD155 gene in human colorectal carcinoma. Gut 2001;49: 236–240.
12. Bevelacqua V, Bevelacqua Y, Candido S, Skarmoutsou E, Amoroso A, Guarneri C, Strazzanti A, Gangemi P, Mazzarino MC, D'Amico F, McCubrey JA, Libra M, and Malaponte G: Nectin like-5 overexpression correlates with the malignant phenotype in cutaneous melanoma. Oncotarget 2012;3:882–892.
13. Nishiwada S, Sho M, Yasuda S, Shimada K, Yamato I, Akahori T, Kinoshita S, Nagai M, Konishi N, and Nakajima Y: Clinical significance of CD155 expression in human pancreatic cancer. Anticancer Res 2015;35:2287–2297.
14. Oshima T, Sato S, Kato J, Ito Y, Watanabe T, Tsuji I, Hori A, Kurokawa T, and Kokubo T: Nectin-2 is a potential target for antibody therapy of breast and ovarian cancers. Mol Cancer 2013;12:60.
15. Asano T, Nanamiya R, Takei J, Nakamura T, Yanaka M, Hosono H, Tanaka T, Sano M, Kaneko MK, and Kato Y: Development of anti-mouse CC chemokine receptor 3 monoclonal antibodies for flow cytometry. Monoclon Antib Immunodiagn Immunother 2021;40:107–112.
16. Asano T, Suzuki H, Tanaka T, Saito M, Li G, Goto N, Nanamiya R, Kaneko MK, and Kato Y: C3Mab-3: A monoclonal antibody for mouse CCR3 for flow cytometry monoclon. Antib Immunodiagn Immunother 2022;41: 74–79.
17. Tanaka T, Nanamiya R, Takei J, Nakamura T, Yanaka M, Hosono H, Sano M, Asano T, Kaneko MK, and Kato Y: Development of anti-mouse CC chemokine receptor 8 monoclonal antibodies for flow cytometry. Monoclon Antib Immunodiagn Immunother 2021;40:65–70.
18. Suzuki H, Saito M, Asano T, Tanaka T, Kitamura K, Kudo Y, Kaneko MK, and Kato Y: C8Mab-3: An anti-mouse CCR8 monoclonal antibody for immunocytochemistry monoclon. Antib Immunodiagn Immunother 2022;41: 110–114.
19. Saito M, Suzuki H, Tanaka T, Asano T, Kaneko MK, and Kato Y: Development of an anti-mouse CCR8 monoclonal antibody (C8Mab-1) for flow cytometry and immunocytochemistry monoclon. Antib Immunodiagn Immunother 2022. DOI: 10.1089/mab.2021.0069.

20. Nanamiya R, Takei J, Asano T, Tanaka T, Sano M, Nakamura T, Yanaka M, Hosono H, Kaneko MK, and Kato Y: Development of anti-human CC chemokine receptor 9 monoclonal antibodies for flow cytometry. Monoclon Antib Immunodiagn Immunother 2021;40:101–106.
21. Kawabata H, Suzuki H, Ohishi T, Kawada M, Kaneko MK, and Kato Y: A defucosylated mouse anti-CD10 monoclonal antibody (31-mG2a-f) exerts antitumor activity in a mouse xenograft model of CD10-overexpressed tumors monoclon. Antib Immunodiagn Immunother 2022;41:59–66.
22. Kawabata H, Ohishi T, Suzuki H, Asano T, Kawada M, Suzuki H, Kaneko MK, and Kato Y: A defucosylated mouse anti-CD10 monoclonal antibody (31-mG2a-f) exerts antitumor activity in a mouse xenograft model of renal cell cancers monoclon. Antib Immunodiagn Immunother 2022. DOI: 10.1089/mab.2021.0049.
23. Yamada S, Kaneko MK, Sayama Y, Asano T, Sano M, Yanaka M, Nakamura T, Okamoto S, Handa S, Komatsu Y, Nakamura Y, Furusawa Y, Takei J, and Kato Y: Development of novel mouse monoclonal antibodies against human CD19. Monoclon Antib Immunodiagn Immunother 2020;39:45–50.
24. Furusawa Y, Kaneko MK, and Kato Y: Establishment of C20Mab-11, a novel anti-CD20 monoclonal antibody, for the detection of B cells. Oncol Lett 2020;20:1961–1967.
25. Furusawa Y, Kaneko MK, and Kato Y: Establishment of an anti-CD20 monoclonal antibody (C20Mab-60) for immunohistochemical analyses. Monoclon Antib Immunodiagn Immunother 2020;39:112–116.
26. Yamada S, Itai S, Nakamura T, Yanaka M, Kaneko MK, and Kato Y: Detection of high CD44 expression in oral cancers using the novel monoclonal antibody, C44Mab-5. Biochem Biophys Rep 2018;14:64–68.
27. Itai S, Fujii Y, Nakamura T, Chang YW, Yanaka M, Saidoh N, Handa S, Suzuki H, Harada H, Yamada S, Kaneko MK, and Kato Y: Establishment of CMab-43, a sensitive and specific anti-CD133 monoclonal antibody, for immunohistochemistry. Monoclon Antib Immunodiagn Immunother 2017;36:231–235.
28. Kaneko MK, Ohishi T, Takei J, Sano M, Nakamura T, Hosono H, Yanaka M, Asano T, Sayama Y, Harada H, Kawada M, and Kato Y: AntiEpCAM monoclonal antibody exerts antitumor activity against oral squamous cell carcinomas. Oncol Rep 2020;44:2517–2526.
29. Hosono H, Ohishi T, Takei J, Asano T, Sayama Y, Kawada M, Kaneko MK, and Kato Y: The anti-epithelial cell adhesion molecule (EpCAM) monoclonal antibody EpMab-16 exerts antitumor activity in a mouse model of colorectal adenocarcinoma. Oncol Lett 2020;20:383.
30. Asano T, Ohishi T, Takei J, Nakamura T, Nanamiya R, Hosono H, Tanaka T, Sano M, Harada H, Kawada M, Kaneko MK, and Kato Y: AntiHER3 monoclonal antibody exerts antitumor activity in a mouse model of colorectal adenocarcinoma. Oncol Rep 2021;46:173.
31. Yamada S, Itai S, Nakamura T, Yanaka M, Chang YW, Suzuki H, Kaneko MK, and Kato Y: Monoclonal antibody L1Mab-13 detected human PD-L1 in lung cancers. Monoclon Antib Immunodiagn Immunother 2018;37:110–115.
32. Furusawa Y, Kaneko MK, Nakamura T, Itai S, Fukui M, Harada H, Yamada S, and Kato Y: Establishment of a monoclonal antibody PMab-231 for tiger podoplanin. Monoclon Antib Immunodiagn Immunother 2019;38:89–95.
33. Furusawa Y, Takei J, Sayama Y, Yamada S, Kaneko MK, and Kato Y: Development of an anti-bear podoplanin monoclonal antibody PMab-247 for immunohistochemical analysis. Biochem Biophys Rep 2019;18:100644.
34. Furusawa Y, Yamada S, Itai S, Nakamura T, Takei J, Sano M, Harada H, Fukui M, Kaneko MK, and Kato Y: Establishment of a monoclonal antibody PMab-233 for immunohistochemical analysis against Tasmanian devil podoplanin. Biochem Biophys Rep 2019;18:100631.
35. Furusawa Y, Yamada S, Itai S, Nakamura T, Yanaka M, Sano M, Harada H, Fukui M, Kaneko MK, and Kato Y: PMab-219: A monoclonal antibody for the immunohistochemical analysis of horse podoplanin. Biochem Biophys Rep 2019;18:100616.
36. Furusawa Y, Yamada S, Itai S, Sano M, Nakamura T, Yanaka M, Fukui M, Harada H, Mizuno T, Sakai Y, Takasu M, Kaneko MK, and Kato Y: PMab-210: A monoclonal antibody against pig podoplanin. Monoclon Antib Immunodiagn Immunother 2019;38:30–36.
37. Furusawa Y, Yamada S, Nakamura T, Sano M, Sayama Y, Itai S, Takei J, Harada H, Fukui M, Kaneko MK, and Kato Y: PMab-235: A monoclonal antibody for immunohistochemical analysis against goat podoplanin. Heliyon 2019;5:e02063.
38. Kaneko MK, Sano M, Takei J, Asano T, Sayama Y, Hosono H, Kobayashi A, Konnai S, and Kato Y: Development and characterization of anti-sheep podoplanin monoclonal antibodies PMab-253 and PMab-260. Monoclon Antib Immunodiagn Immunother 2020;39:144–155.
39. Kato Y, Furusawa Y, Itai S, Takei J, Nakamura T, Sano M, Harada H, Yamada S, and Kaneko MK: Establishment of an anticetacean podoplanin monoclonal antibody PMab-237 for immunohistochemical analysis. Monoclon Antib Immunodiagn Immunother 2019;38:108–113.
40. Kato Y, Furusawa Y, Sano M, Takei J, Nakamura T, Yanaka M, Okamoto S, Handa S, Komatsu Y, Asano T, Sayama Y, and Kaneko MK: Development of an anti-sheep podoplanin monoclonal antibody PMab-256 for immunohistochemical analysis of lymphatic endothelial cells. Monoclon Antib Immunodiagn Immunother 2020;39:82–90.
41. Kato Y, Furusawa Y, Yamada S, Itai S, Takei J, Sano M, and Kaneko MK: Establishment of a monoclonal antibody PMab-225 against alpaca podoplanin for immunohistochemical analyses. Biochem Biophys Rep 2019;18:100633.
42. Kato Y, Yamada S, Furusawa Y, Itai S, Nakamura T, Yanaka M, Sano M, Harada H, Fukui M, and Kaneko MK: PMab-213: A monoclonal antibody for immunohistochemical analysis against pig podoplanin. Monoclon Antib Immunodiagn Immunother 2019;38:18–24.
43. Takei J, Yamada S, Konnai S, Ishinazaka T, Shimozuru M, Kaneko MK, and Kato Y: PMab-241 specifically detects bear podoplanin of lymphatic endothelial cells in the lung of brown bear. Monoclon Antib Immunodiagn Immunother 2019;38:282–284.
44. Tanaka T, Asano T, Sano M, Takei J, Hosono H, Nanamiya R, Nakamura T, Yanaka M, Harada H, Fukui M, Suzuki H, Uchida K, Nakagawa T, Kato Y, and Kaneko MK: Development of monoclonal antibody PMab-269 against california sea lion podoplanin. Monoclon Antib Immunodiagn Immunother 2021;40:124–133.
45. Yamada S, Itai S, Nakamura T, Yanaka M, Saidoh N, Chang YW, Handa S, Harada H, Kagawa Y, Ichii O, Konnai S, Kaneko MK, and Kato Y: PMab-52: Specific and

sensitive monoclonal antibody against cat podoplanin for immunohistochemistry. Monoclon Antib Immunodiagn Immunother 2017;36:224–230.
46. Goto N, Suzuki H, Tanaka T, Asano T, Kaneko MK, and Kato Y: Development of a monoclonal antibody PMab-292 against ferret podoplanin. Monoclon Antib Immunodiagn Immunother 2022;41:101–109.
47. Sayama Y, Kaneko MK, and Kato Y: Development and characterization of TrMab6, a novel antiTROP2 monoclonal antibody for antigen detection in breast cancer. Mol Med Rep 2021;23:92.
48. Sayama Y, Kaneko MK, Takei J, Hosono H, Sano M, Asano T, and Kato Y: Establishment of a novel anti-TROP2 monoclonal antibody TrMab-29 for immunohistochemical analysis. Biochem Biophys Rep 2021;25:100902.
49. Takei J, Asano T, Nanamiya R, Nakamura T, Yanaka M, Hosono H, Tanaka T, Sano M, Kaneko MK, Harada H, and Kato Y: Development of anti-human T cell immunoreceptor with Ig and ITIM domains (TIGIT) monoclonal antibodies for flow cytometry. Monoclon Antib Immunodiagn Immunother 2021;40:71–75.
50. Saito M, Harigae Y, Li G, Asano T, Tanaka T, Suzuki H, Kaneko MK, and Kato Y: C_3Mab-2: An anti-mouse CCR3 monoclonal antibody for immunocytochemistry. Monoclon Antib Immunodiagn Immunother 2022;41:45–49.
51. Saito M, Tanaka T, Asano T, Nakamura T, Yanaka M, Handa S, Komatsu Y, Harigae Y, Tateyama N, Nanamiya R, Li G, Suzuki H, Kaneko MK, and Kato Y: C8Mab-2: An anti-mouse C–C motif chemokine receptor 8 monoclonal antibody for immunocytochemistry monoclon. Antib Immunodiagn Immunother 2022;41:115–119.
52. Saito M, Suzuki H, Harigae Y, Li G, Tanaka T, Asano T, Kaneko MK, and Kato Y: C9Mab-1: An anti-mouse CCR9 monoclonal antibody for immunocytochemistry. Monoclon Antib Immunodiagn Immunother 2022;41:120–124.
53. Chauvin JM, Pagliano O, Fourcade J, Sun Z, Wang H, Sander C, Kirkwood JM, Chen TH, Maurer M, Korman AJ, and Zarour HM: TIGIT and PD-1 impair tumor antigen-specific $CD8^+$ T cells in melanoma patients. J Clin Invest 2015;125:2046–2058.
54. Dixon KO, Schorer M, Nevin J, Etminan Y, Amoozgar Z, Kondo T, Kurtulus S, Kassam N, Sobel RA, Fukumura D, Jain RK, Anderson AC, Kuchroo VK, and Joller N: Functional anti-TIGIT antibodies regulate development of autoimmunity and antitumor immunity. J Immunol 2018; 200:3000–3007.
55. Johnston RJ, Comps-Agrar L, Hackney J, Yu X, Huseni M, Yang Y, Park S, Javinal V, Chiu H, Irving B, Eaton DL, and Grogan JL: The immunoreceptor TIGIT regulates antitumor and antiviral $CD8^+$ T cell effector function. Cancer Cell 2014;26:923–937.
56. Freed-Pastor WA, Lambert LJ, Ely ZA, Pattada NB, Bhutkar A, Eng G, Mercer KL, Garcia AP, Lin L, Rideout WM, 3rd, Hwang WL, Schenkel JM, Jaeger AM, Bronson RT, Westcott PMK, Hether TD, Divakar P, Reeves JW, Deshpande V, Delorey T, Phillips D, Yilmaz OH, Regev A, and Jacks T: The CD155/TIGIT axis promotes and maintains immune evasion in neoantigen-expressing pancreatic cancer. Cancer Cell 2021;39:1342.e14–1360.e14.

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: March 4, 2022
Accepted: April 19, 2022