MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 32, Number 6, 2013

DOI: 10.1089/mab.2013.0050

Original Articles

Multi-Specific Monoclonal Antibody MsMab-2 Recognizes IDH1-R132L and IDH2-R172M Mutations

Satoshi Ogasawara,* Mika Kato Kaneko,* Yuta Tsujimoto,* Xing Liu, and Yukinari Kato

Mutations of isocitrate dehydrogenase 1/2 (IDH1/2) produce oncometabolite R(-)-2-hydroxyglutarate in several tumors. Arginine 132 (R132) of IDH1 and arginine 172 (R172) of IDH2 are functionally important residues. Although MsMab-1 monoclonal antibody (MAb), which is multi-specific for mutated IDH1/2, has been established, MsMab-1 does not react with all IDH1/2 mutations. Herein, we immunized rats with IDH1-R132L peptide, and screened IDH1-R132L-reactive/IDH1-wild-type non-reactive MAbs in enzyme-linked immunosorbent assay. Unexpectedly, the newly established MsMab-2 MAb recognized not only IDH1-R132L but also IDH2-R172M in Western blot analyses, neither of which was detected by MsMab-1. Taken together, the combination of MsMab-1 and MsMab-2 could be useful in diagnosis of mutated IDH1/2-bearing tumors.

Introduction

Isocitrate dehydrogenase 1/2 (IDH1/2) catalyzes the oxidative carboxylation of isocitrate to α-ketoglutarate.[1] In contrast, somatic mutations of IDH1/2 were found to convert α-ketoglutarate to oncometabolite R(-)-2-hydroxyglutarate (2-HG). Most changes by far are heterozygous. A recent report describes two gliomas that lost the wild-type IDH1 allele but retained the mutant IDH1 following tumor progression from anaplastic astrocytoma to glioblastoma.[2] The intratumoral 2-HG level was 14-fold lower in the glioblastomas lacking wild-type IDH1 compared with glioblastomas of heterozygous IDH1 mutations, indicating that both wild-type and mutant IDH1 alleles are necessary for 2-HG production in glioma cells. It is noteworthy that the expression of mitochondrial IDH2 mutations led to robust 2-HG production in a manner that is independent of wild-type mitochondrial IDH function.[3] Another report demonstrated that 2-HG can inhibit histone demethylation and that inhibition of histone demethylation can be sufficient to block the differentiation of non-transformed cells.[4] Turcan and colleagues also showed that IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype.[5] Taken together, the blockade of 2-HG production by inhibiting the functional mutated IDH1/2 is thought to be an ideal molecular targeting therapy against gliomas and leukemias.[6,7]

The IDH1 mutations are remarkably specific to a single codon in the conserved and functionally important arginine 132 residue (R132).[8] In contrast, the IDH2 mutations are specific to a single codon in arginine 172 residue (R172). IDH2 mutations of leukemias were discovered subsequently in arginine 140 residue (R140), which is found more frequently than R172.[9] In gliomas, IDH1 mutations were reported as IDH1-R132H (664/716, 92.7%), IDH1-R132C (29/716, 4.2%), IDH1-R132S (11/716, 1.5%), IDH1-R132G (10/716, 1.4%), and IDH1-R132L (2/716, 0.2%).[10] In contrast, IDH2 mutations were reported as IDH2-R172K (20/31, 64.5%), IDH2-R172M (6/31, 19.3%), and IDH2-R172W (5/31, 16.2%) in gliomas. To date, we have established several mono-specific monoclonal antibodies (MAbs) against IDH1/2 mutations.[11–17] A recent report has described a multi-specific anti-mutated IDH1/2 monoclonal antibody, MsMab-1, which is useful for diagnosis of IDH1/2 mutation-bearing gliomas.[18,19] Herein, we report another multi-specific MAb, MsMab-2, which can react with MsMab-1-negative IDH1/2 mutations.

Materials and Methods

Cell lines

Chinese hamster ovary (CHO)-K1 and Sp2/0-Ag14 were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and was cultured at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in RPMI 1640 medium, including 2 mM L-glutamine (Nacalai Tesque, Kyoto, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Life Technologies, Carlsbad, CA).

Hybridoma production

MsMab-2 was produced using rat medial iliac lymph node methods.[18] The institutional animal committee approved our study. Briefly, WKY/Izm rats (Japan SLC Inc., Shizuoka, Japan) were immunized by injecting 170 μg of synthetic peptides of CGGVKPIIIGLHAYGDQYRA (IDH1-R132L), conjugated with KLH together with Freund's complete adjuvant

Department of Regional Innovation, Tohoku University Graduate School of Medicine, Aoba-ku, Sendai, Miyagi, Japan.
*These authors contributed equally to this work.

(FCA, Sigma-Aldrich, St. Louis, MO) into the footpad. The lymphocytes were fused with mouse myeloma Sp2/0-Ag14 cells using polyethylene glycol (PEG). The culture supernatants were screened using ELISA for binding to the IDH1-R132L and IDH1-WT peptides, conjugated with BSA.

Enzyme-linked immunosorbent assay

Synthetic peptides or recombinant proteins were immobilized on Nunc Maxisorp 96-well immunoplates (Thermo Fisher Scientific, Waltham, MA) at 1 μg/mL for 30 min. Synthetic peptides are as follows: GGVKPIIIGRHAYGDQYRA (IDH1-WT), GGVKPIIIGHHAYGDQYRA (IDH1-R132H), GGVKPIIIGCHAYGDQYRA (IDH1-R132C), GGVKPIIIGSHAYGDQYRA (IDH1-R132S), GGVKPIIIGGHAYGDQYRA (IDH1-R132G), GGVKPIIIGLHAYGDQYRA (IDH1-R132L), and GGVKPIIIGVHAYGDQYRA (IDH1-R132V). After blocking with SuperBlock T20 Blocking Buffer (Thermo Fisher Scientific), the plates were incubated with culture supernatant or purified MAbs (1 μg/mL) with subsequent 1:1000 diluted peroxidase-conjugated anti-rat IgG or anti-mouse IgG (Dako, Glostrup, Denmark). The enzymatic reaction was conducted with 1-Step Ultra TMB-ELISA (Thermo Fisher Scientific). The optical density was measured at 655 nm using an iMark microplate reader (Bio-Rad Laboratories, Philadelphia, PA). These reactions were performed with a volume of 50 μL at 37°C.

Plasmid preparation

Human IDH1 cDNA (GenBank accession no. AF113917) and human IDH2 cDNA (accession no. NM_002168) encoding a full-length open-reading frame (ORF) were obtained using PCR with a human lung cDNA library (Cosmobio Co., Tokyo, Japan) and cDNA derived from the U373 glioblastoma cell line as template, respectively. The primer set for IDH1 was: EcoRI-IDH1-F1, 5′-cacgaattcATGTCCAAAAAAATCA GTGG-3′ and SalI-IDH1-R1, 5′-gtggtcgacTTAAAGTTTGGC CTGAGCTA-3′; and for IDH2: EcoRI-IDH2.F1, 5′-ccgaattc gggATGGCCGGCTACCTGCGGG-3′ and SalI-IDH2woterR1359, 5′-gccgtcgacCTACTGCCTGCCCAGGGCTCT-3′. The amplified cDNA was subcloned into a pcDNA3.1/V5-His-TOPO vector (Life Technologies). Substitution of the arginine 132 (R132) to appropriate amino acid codons in IDH1 or arginine 172 (R172) to appropriate amino acid codons in IDH2 was done using the QuikChange Lightning site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA). The full-length IDH1 was subcloned into an expression vector, pMAL-c2 (New England Biolabs, Beverly, MA), via EcoRI and SalI restriction sites. The full-length IDH2 and each mutated ORF were amplified using the primer set EcoRI-IDH2.F1 and IDH2woterR1356-XhoI, 5′-taactcgagcgCTGCC TGCCCAGGGCTCTG-3′. These PCR products were digested with EcoRI and XhoI restriction enzymes and were subcloned into pcDNA3 vector (Life Technologies) together with the nucleotide sequence (ctcgagTGGCGTTGCCATGCCAGGT GCCGAAGATGATGTGGTGTAAtctaga), which encodes 12 amino acids, GVAMPGAEDDVV (PA tag).[13,16,18,19]

Protein expression using bacteria cells and mammalian cells

Competent *Escherichia coli* TOP-10 cells (Life Technologies) were transformed with the plasmid pMAL-IDH1-WT, pMAL-IDH1-R132H, pMAL-IDH1-R132C, pMAL-IDH1-R132S, pMAL-IDH1-R132G, pMAL-IDH1-R132L, or pMAL-IDH1-R132V. Then they were cultured overnight at 37°C in LB medium (Life Technologies) containing 100 μg/mL ampicillin (Sigma-Aldrich). Cell pellets were resuspended in phosphate-buffered saline (PBS) with 1% Triton X-100 with 50 μg/mL aprotinin (Sigma-Aldrich). After sonication using Branson Advanced Sonifier (Branson Ultrasonics, Danbury, CT), the crude extracts were collected by centrifugation (9000 *g*, 30 min, 4°C). The supernatants were loaded onto amylose resin (New England Biolabs). The loaded resins were washed extensively with column buffer (20 mM Tris-HCl [pH 7.4], 200 mM NaCl, 1 mM EDTA). The fusion proteins were eluted by column buffer with 10 mM maltose. Then CHO cells were transfected with appropriate amounts of plasmids (pcDNA3.1/IDH1-WT, pcDNA3.1/IDH1-R132H, pcDNA3.1/IDH1-R132C, pcDNA3.1/IDH1-R132S, pcDNA3.1/IDH1-R132G, pcDNA3.1/IDH1-R132L, pcDNA3.1/IDH1-R132V, pcDNA3/IDH2-R172K, pcDNA3/IDH2-R172M, pcDNA3/IDH2-R172W, pcDNA3/IDH2-R172G, pcDNA3/IDH2-R172S) using Lipofectamine LTX (Life Technologies) according to the manufacturer's instructions. The expression level of IDH1/2 was confirmed using Western blot analysis.

Western blot analyses

Cultured cell pellets were lysed with PBS with 1% TritonX-100 for 30 min on ice. The lysate supernatants were centrifuged for 15 min at 15,000 rpm to remove cellular debris. Cell lysates were prepared for Western blot analyses by boiling in SDS sample buffer (Nacalai Tesque). They were electrophoresed on 5–20% polyacrylamide gels (Wako Pure Chemical Industries). The separated proteins were transferred to a PVDF membrane (EMD Millipore, Billerica, MA). After blocking with 4% skim milk in PBS with 0.05% Tween-20 for 15 min, the membrane was incubated with 1 μg/mL of primary antibodies for 30 min. Then the membrane was incubated with peroxidase-conjugated secondary antibodies (1:1000 diluted; Dako) for 15 min and developed with ImmunoStar LD Chemiluminescence Reagent (Wako Pure Chemical Industries) using Sayaca-Imager (DRC Co., Tokyo, Japan).

Results and Discussion

Production of an IDH1-R132L-specific monoclonal antibody

We previously established several mono-specific anti-mutated IDH1/2 MAbs: HMab-1 against IDH1-R132H, SMab-1 against IDH1-R132S, GMab-r1 against IDH1-R132G, KMab-1 against IDH2-R172K, MMab-1 against IDH2-R172M, and WMab-1 against IDH2-R172W.[11–17] Recently we reported a multi-specific MAb MsMab-1 that recognizes IDH1-R132H, IDH1-R132S, IDH1-R132G, and IDH2-R172M, but not IDH1/2 wild type of mammalian cells in Western blot analyses.[18] Furthermore, MsMab-1 is very useful in immunohistochemistry against mutated IDH1/2-possessing gliomas. However, MAbs against other IDH1/2 mutations, including IDH1-R132L, are still necessary to cover all IDH1/2 mutations in immunohistochemistry. Here, we immunized rats with synthetic peptides of IDH1-R132L and screened IDH1-R132L-reactive/IDH1 wild-type non-reactive MAbs using ELISA.

After limiting dilution, clone MsMab-2 (rat IgG_{2a}, kappa) against IDH1-R132L was established.

Specificity of multi-specific MAbs against IDH1 peptides and proteins in ELISA

The specificity of MsMab-2 and MsMab-1 was investigated by ELISA using synthetic IDH1 peptides (IDH1-WT, IDH1-R132H, IDH1-R132C, IDH1-R132S, IDH1-R132G, IDH1-R132L, IDH1-R132V) or purified IDH1 proteins expressed in *Escherichia coli*. As depicted in Figure 1A, MsMab-2 strongly reacted with IDH1-R132L peptide, moderately with IDH1-R132S, IDH1-R132G, and IDH1-R132V peptides, and very weakly with IDH1-R132H peptide. In contrast, MsMab-2 weakly recognized IDH1-R132S, IDH1-R132G, and IDH1-R132L recombinant proteins. Compatible with previous reports, MsMab-1 strongly recognized IDH1-R132H, IDH1-R132S, and IDH1-R132G peptides and weakly with IDH1-R132C and IDH1-R132L peptides (Fig. 1B). Recombinant proteins of IDH1-R132S and IDH1-R132G were detected strongly by MsMab-1, although the reaction against IDH1-R132H was slightly better compared with wild-type IDH1. When the concentration of coated IDH1-WT peptide or protein was increased or the concentration of MsMab-1 MAb was increased in ELISA, the reactivity of MsMab-1 against IDH1-WT was also augmented (data not shown), demonstrating that the concentration of MsMab-1 MAb is very critical in immunohistochemistry to detect only IDH1/2 mutations. These results indicate that MsMab-2 is a multi-specific MAb against several IDH1 mutations, although MsMab-2 was produced by immunizing IDH1-R132L peptide.

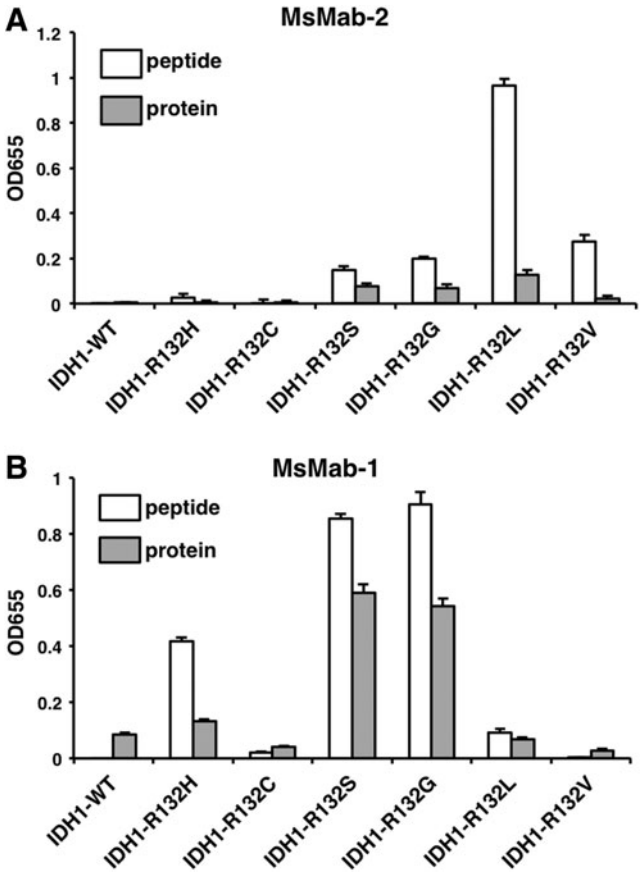

FIG. 1. Reactivity of multi-specific antibodies against IDH1 peptides and proteins by ELISA. Synthetic peptides or purified proteins of IDH1 wild type (WT) and mutants (R132H, R132C, R132S, R132G, R132L, R132V) expressed in *E. coli* were immobilized, followed by MsMab-2 (**A**) and MsMab-1 (**B**). Data are representative of mean ± S.D. of triplicates.

Specificity of anti-mutated IDH MAbs in Western blot analyses

To further determine the specificity of MsMab-2 against IDH1/2 mutations, IDH1/2-WTs and IDH1/2 mutants (IDH1-R132H, IDH1-R132C, IDH1-R132S, IDH1-R132G, IDH1-R132L, IDH1-R132V, IDH2-R172K, IDH2-R172M, IDH2-R172W, IDH2-R172G, IDH2-R172S) were transiently expressed in CHO cells and were Western blotted using MsMab-2 and other anti-IDH1/2 MAbs. As shown in Figure 2, MsMab-2 recognized not only IDH1-R132L protein but also IDH2-R172M protein. Unexpectedly, the reactivity of MsMab-2 against IDH2-R172M was much stronger than that against IDH1-R132L. In contrast, MsMab-1 strongly reacted with IDH1-R132S, IDH1-R132G, IDH2-R172G, and IDH2-R172S; moderately with IDH1-R132H; and weakly with IDH2-R172M, indicating that MsMab-2 epitope is totally different from MsMab-1 epitope. Recombinant human IDH1 and endogenous hamster IDH1 proteins were detected by RcMab-1 MAb. In contrast, only IDH2-WT and IDH2-R172K were recognized by KrMab-3. These results indicate that only one

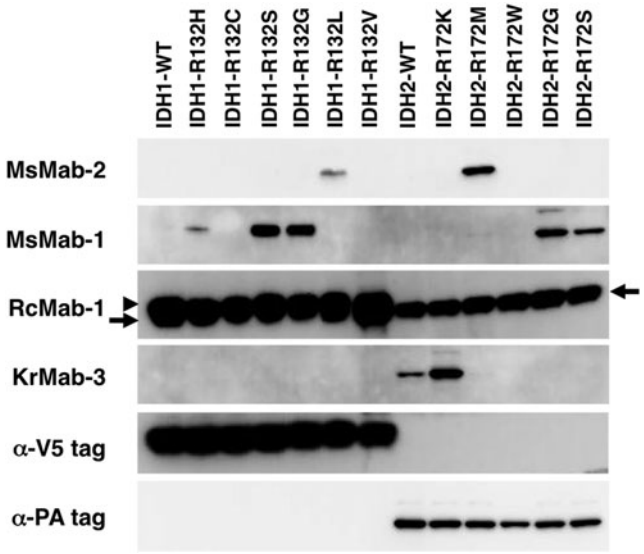

FIG. 2. Western blot analyses by anti-IDH1/2 MAbs against wild-type and mutated IDH1/2 proteins. Total cell lysate from CHO cells expressing IDH1 (lane 1, IDH1-WT; lane 2, IDH1-R132H; lane 3, IDH1-R132C; lane 4, IDH1-R132S, lane 5, IDH1-R132G; lane 6, IDH1-R132L; lane 7, IDH1-R132V) and IDH2 (lane 8, IDH2-WT; lane 9, IDH2-R172K; lane 10, IDH2-R172M; lane 11, IDH2-R172W; lane 12, IDH2-R172G; lane 13, IDH2-R172S) were electrophoresed under reducing conditions using 5–20% gels and were Western blotted with MsMab-2, MsMab-1, RcMab-1, KrMab-3, anti-V5 tag, and anti-PA tag. RcMab-1 detected two bands, including recombinant human IDH1 (arrowhead) and endogenous hamster IDH1 (arrow).

mutation at codon R132 of IDH1 or R172 of IDH2 makes conformational variation of IDH1/2 proteins.

In conclusion, the newly established MsMab-2 is a multi-specific MAb that recognizes IDH1-R132L and IDH2-R172M of mammalian cells. The combination of MsMab-2 and MsMab-1 covers more than 95% of IDH1 mutations, which could be extremely useful for diagnosis and biological evaluation of IDH1/2 mutation-bearing tumors. Unfortunately, we could not obtain an IDH1-R132L or IDH2-R172M-possessing specimen in this study; therefore, we could not show clearly the usefulness of MsMab-2 in immunohistochemistry. Nevertheless, MsMab-2 is believed to be useful in immunohistochemistry because MsMab-2 is able to react strongly with recombinant proteins expressed in mammalian cells (Fig. 2). The combination of multi-specific MAbs with previously established anti-mutated IDH2 mono-specific MAbs (KMab-1 and WMab-1) covers almost all IDH2 mutations, and could increase the diagnostic rate of IDH1/2 mutation-bearing tumors.

Acknowledgments

We thank Takuro Nakamura and Noriko Saidoh for their excellent technical assistance. This work was supported in part by the Platform for Drug Discovery, Informatics, and Structural Life Science (Y.K., M.K.K., S.O.), by Regional Innovation Strategy Support Program (Y.K.), by KAKENHI (25462242): Grant-in-Aid for Scientific Research (C) (Y.K.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by Office for Women Researchers of Tohoku University (M.K.K.), and by Intelligent Cosmos Academic Foundation (Y.K.).

Author Disclosure Statement

The authors have no financial interests to disclose.

References

1. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, and Su SM: Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 2009;462:739–744.
2. Jin G, Reitman ZJ, Duncan CG, Spasojevic I, Gooden DM, Rasheed BA, Yang R, Lopez GY, He Y, McLendon RE, Bigner DD, and Yan H: Disruption of wild-type IDH1 suppresses D-2-hydroxyglutarate production in IDH1-mutated gliomas. Cancer Res 2013;73:496–501.
3. Ward PS, Lu C, Cross JR, Abdel-Wahab O, Levine RL, Schwartz GK, and Thompson CB: The potential for isocitrate dehydrogenase mutations to produce 2-hydroxyglutarate depends on allele specificity and subcellular compartmentalization. J Biol Chem 2013;288:3804–3815.
4. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Khanin R, Figueroa ME, Melnick A, Wellen KE, O'Rourke DM, Berger SL, Chan TA, Levine RL, Mellinghoff IK, and Thompson CB: IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature 2012;483:474–478.
5. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS, Thompson CB, Kaufman A, Guryanova O, Levine R, Heguy A, Viale A, Morris LG, Huse JT, Mellinghoff IK, and Chan TA: IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 2012;483:479–483.
6. Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, Tsoi J, Clark O, Oldrini B, Komisopoulou E, Kunii K, Pedraza A, Schalm S, Silverman L, Miller A, Wang F, Yang H, Chen Y, Kernytsky A, Rosenblum MK, Liu W, Biller SA, Su SM, Brennan CW, Chan TA, Graeber TG, Yen KE, and Mellinghoff IK: An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science 2013;340:626–630.
7. Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, Straley K, Kernytsky A, Liu W, Gliser C, Yang H, Gross S, Artin E, Saada V, Mylonas E, Quivoron C, Popovici-Muller J, Saunders JO, Salituro FG, Yan S, Murray S, Wei W, Gao Y, Dang L, Dorsch M, Agresta S, Schenkein DP, Biller SA, Su SM, de Botton S, and Yen KE: Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. Science 2013;340:622–626.
8. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, and Bigner DD: IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765–773.
9. Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Kronke J, Bullinger L, Spath D, Kayser S, Zucknick M, Gotze K, Horst HA, Germing U, Dohner H, and Dohner K: IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. J Clin Oncol 2010;28:3636–3643.
10. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, Weller M, Herold-Mende C, Unterberg A, Jeuken JW, Wesseling P, Reifenberger G, and von Deimling A: Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol 2009;118:469–474.
11. Kato Y, Jin G, Kuan CT, McLendon RE, Yan H, and Bigner DD: A monoclonal antibody IMab-1 specifically recognizes IDH1R132H, the most common glioma-derived mutation. Biochem Biophys Res Commun 2009;390:547–551.
12. Kato Y, Natsume A, and Kaneko MK: A novel monoclonal antibody GMab-m1 specifically recognizes IDH1-R132G mutation. Biochem Biophys Res Commun 2013;432:564–567.
13. Kato Y, and Kaneko MK: Generation of a novel monoclonal antibody WMab-1 specific for IDH2-R172W mutation. Biochem Biophys Res Commun 2013;433:374–378.
14. Kaneko MK, Tsujimoto Y, Hozumi Y, Goto K, and Kato Y: Novel monoclonal antibodies GMab-r1 and LMab-1 specifically recognize IDH1-R132G and IDH1-R132L mutations. Monoclon Antib Immunodiagn Immunother 2013;32:224–228.
15. Kaneko MK, Tian W, Takano S, Suzuki H, Sawa Y, Hozumi Y, Goto K, Yamazaki K, Kitanaka C, and Kato Y: Establishment of a novel monoclonal antibody SMab-1 specific for IDH1-R132S mutation. Biochem Biophys Res Commun 2011;406:608–613.
16. Kaneko MK, Morita S, Tsujimoto Y, Yanagiya R, Nasu K, Sasaki H, Hozumi Y, Goto K, Natsume A, Watanabe M, Kumabe T, Takano S, and Kato Y: Establishment of novel monoclonal antibodies KMab-1 and MMab-1 specific for IDH2 mutations. Biochem Biophys Res Commun 2013;432:40–45.

17. Takano S, Kato Y, Yamamoto T, Kaneko MK, Ishikawa E, Tsujimoto Y, Matsuda M, Nakai K, Yanagiya R, Morita S, Tsuboi K, and Matsumura A: Immunohistochemical detection of IDH1 mutation, p53, and internexin as prognostic factors of glial tumors. J Neurooncol 2012;108:361–373.
18. Kaneko M, Ogasawara S, and Kato Y: Establishment of a novel multi-specific monoclonal antibody MsMab-1 recognizing both IDH1 and IDH2 mutations. Tohoku J Exp Med 2013;230:103–109.
19. Liu X, Kato Y, Kaneko MK, Sugawara M, Ogasawara S, Tsujimoto Y, Naganuma Y, Yamakawa M, Tsuchiya T, and Takagi M: Isocitrate dehydrogenase 2 mutation is a frequent event in osteosarcoma detected by a multi-specific monoclonal antibody MsMab-1. Cancer Med in press: DOI: 10.1002/cam4.149.

Address correspondence to:
Dr. Yukinari Kato
Department of Regional Innovation
Tohoku University Graduate School of Medicine
2-1 Seiryo-machi
Aoba-ku, Sendai
Miyagi 980-8575
Japan

E-mail: yukinari-k@bea.hi-ho.ne.jp

yukinarikato@med.tohoku.ac.jp

Received: July 9, 2013
Accepted: July 29, 2013