



Short Communication

Immunohistochemistry using monoclonal antibody MsMab-2 is useful to detect IDH1 R132L in intrahepatic cholangiocarcinomaAkimasa Hayashi,^{1†} Kento Misumi,^{1†} Junji Shibahara,¹ Norihiro Kokudo,² Yukinari Kato³ and Masashi Fukayama¹¹Department of Pathology, Graduate School of Medicine, ²Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo and ³Department of Regional Innovation, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Immunohistochemical analysis using specific antibodies is a useful and convenient method to detect proteins altered by somatic mutations. We previously generated the rat monoclonal antibody MsMab-2, which recognizes isocitrate dehydrogenase (IDH)1 R132L and IDH2 R172M. In the present study, we used an immunohistochemical method to examine MsMab-2 immunoreactivity in 95 cases of intrahepatic cholangiocarcinoma, including five IDH1 R132L and one IDH2 R172M mutant cases confirmed by direct sequencing. Tissue microarray section slides of all IDH1/2-mutant and wild-type cases, as well as whole section slides of IDH1 R132L and IDH2 R172M cases were immunostained using an autostainer. All IDH1 R132L cases showed positive staining for MsMab-2, while other IDH1/2 mutant and IDH1/2 wild-type cases were negative. Tumor cells of the immunopositive cases invariably showed strong reactivity using whole-section slides. We consider immunohistochemical analysis using MsMab-2 to be a useful means of detecting IDH1 R132L. Further analysis of its effectiveness against IDH2 R172M is necessary because of the small sample size in this study.

Key words: IDH, immunohistochemistry, intrahepatic cholangiocarcinoma, MsMab-2

Cholangiocarcinoma is a malignant biliary neoplasm with a generally poor patient prognosis, which appears to be increasing in overall incidence.¹ Recent genome-wide studies have revealed genomic alternations including somatic mutations of genes such as isocitrate dehydrogenases 1/2 (*IDH1/2*), *KRAS*, *BRC*A associated protein-1 (*BAP1*), AT-rich interactive domain 1 A (*ARID1A*), and Polybromo 1 (*PBRM1*).^{2,3}

In genes with mutation hot spots that dramatically alter the functioning of their encoded proteins, immunohistochemical

analysis with specific antibodies is useful to detect protein alterations. For example, the *BRAF* mutation status can be detected easily and precisely using a monoclonal antibody against *BRAF* V600E.⁴ *IDH1* and *IDH2* are also good candidates because they each have a mutation hot spot, in *IDH1* exon 4 at codon 132 and in *IDH2* exon 4 at codon 172, as confirmed in several previous studies^{2,3,5,6} and the Catalogue of Somatic Mutations in Cancer (COSMIC) (<http://cancer.sanger.ac.uk/cosmic>). Out of four major mutations and resultant alterations in *IDH1*, previous studies succeeded in immunohistochemically detecting IDH1 R132H,⁷ but none could detect IDH1 R132L.

In the present study, we focused on IDH1 R132L and the usefulness of immunohistochemical analysis with the rat monoclonal antibody MsMab-2 which recognizes IDH1 R132L and IDH2 R172M. We previously generated this antibody, and examined its reliability and specificity using western blotting and enzyme-linked immunosorbent assays,⁸ but did not confirm this with an immunohistochemical assay using formalin-fixed, paraffin-embedded (FFPE) samples. Although IDH1 R132L has been identified in just 1 % of cholangiocarcinoma cases,^{9,10} our mutational analysis showed that it was present in 5 % of cases (5/95) of intrahepatic cholangiocarcinoma (ICC). In this study we aimed not only to confirm the sensitivity and specificity of MsMab-2 using FFPE surgical samples, but also to examine its usefulness in the routine diagnosis of ICC.

MATERIALS AND METHODS**Patients and tissue microarrays**

A total of 95 patients with primary ICC who underwent surgical treatment at The University of Tokyo Hospital from January 1, 1995 to December 31, 2013 were enrolled in this study. Pathology reports and all tissue slides were reviewed for all patients to confirm the diagnoses. Distal (extrahepatic)

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and perihilar cholangiocarcinomas and intraductal papillary mucinous neoplasms were excluded.

Tissue microarrays (TMAs) were generated for all 95 ICC cases according to well-established procedures. In brief, two tissue cores (2 mm diameter each) were punched out of each donor paraffin block and transferred to each of the recipient TMA blocks.

IDH1/2 mutational status

The *IDH1/2* mutational status was analyzed in our previous study.¹¹ In short, tumor DNA was extracted from FFPE tissue blocks and amplified by PCR with paired primers focusing on exon 4 at codon 132 of *IDH1* and exon 4 at codon 172 of *IDH2*. Amplified DNA was analyzed using direct sequencing.

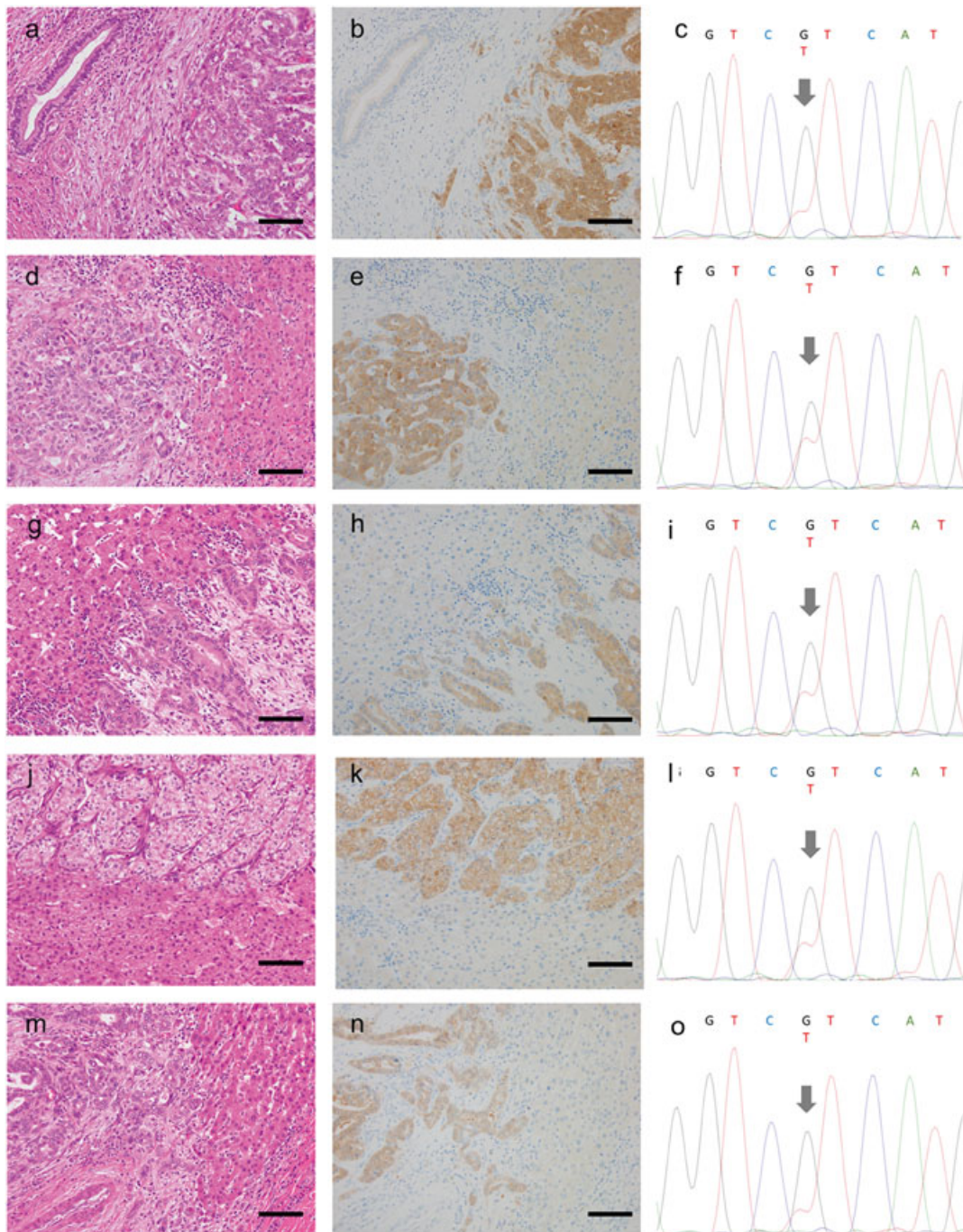


Figure 1 Representative photomicrographs of immunohistochemistry analysis using MsMab-2 in five ICC cases with IDH R132L. Images of H&E staining (a, d, g, j, and m) and immunohistochemical staining using MsMab-2 (b, e, h, k, and n), as well as results of sequencing analyses (c, f, i, l, and o), are shown for cases of ICC with IDH R132L. Each column shows data from the same case. Scale bar represents 100 μ m.

IDH1 R132 mutations were observed in 19 of 95 (20.0 %) ICC cases, including five with *IDH1* R132L, 11 with *IDH1* R132C, and three with *IDH1* R132G. *IDH2* R172 mutations were confirmed in just two cases (2.1 %), including one with *IDH2* R172M and one with *IDH2* R172K.

Immunohistochemistry Using the Monoclonal Antibody MsMab-2

The rat monoclonal antibody MsMab-2, shown to recognize *IDH1* R132L and *IDH2* R172M in a previous Western blotting analysis, was generated previously.⁸ TMA sections (3 μ m-thick), including both *IDH1/2* wild-type and mutant cases,

were subjected to immunohistochemical staining using a Ventana BenchMark XT automated immunostainer (Roche, Basel, Switzerland). Whole tissue sections of representative areas of tumors with *IDH1* R132L and *IDH2* R172M were immunostained using the same protocols.

Ethics

The University of Tokyo Medical Research Center Ethics Committee approved the study. Clinical samples were collected following written informed consent from patients under The University of Tokyo Institutional guidelines for the study of human tissues.

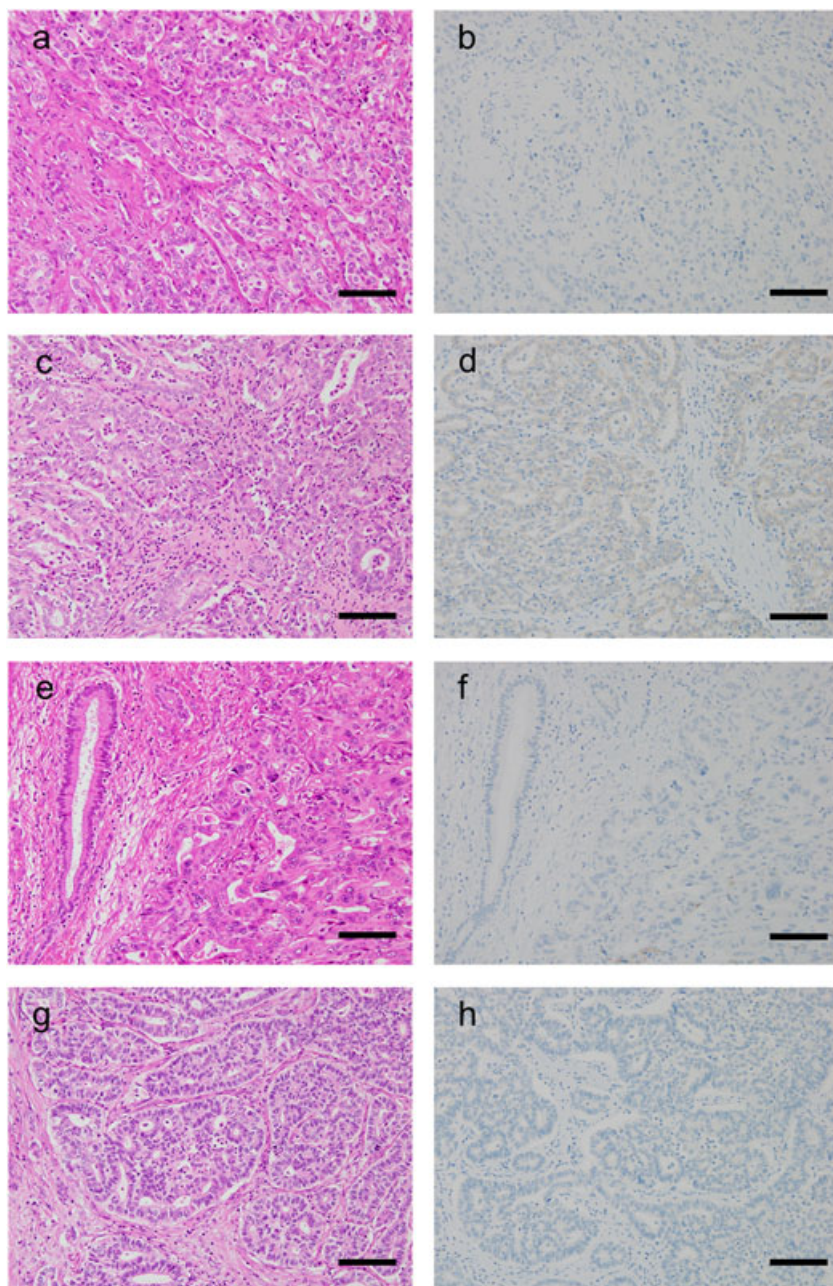


Figure 2 Representative photomicrographs of immunohistochemistry analysis using MsMab-2 in ICC with *IDH1* R132C, *IDH1* R132G, *IDH2* R172M, and wild-type *IDH 1/2*. Images of H&E staining (**a**, **c**, **e**, and **g**) and immunohistochemical staining using MsMab-2 (**b**, **d**, **f**, and **h**) are shown for cases of ICC with *IDH1* R132C (**a**, **b**), *IDH1* R132G (**c**, **d**), *IDH2* R172M (**e**, **f**), and wild-type *IDH 1/2* (**g**, **h**). Scale bar represents 100 μ m.

RESULTS

Detection of IDH1 R132L Using MsMab-2

All IDH1 R132L mutant cases showed immunohistochemical positivity for MsMab-2, while other IDH1 R132 mutant and wild-type cases had no immunoreactivity (Fig. 1, Fig. 2, and Table 1). Tumor cells invariably showed strong immunoreactivity to MsMab-2 in the analysis of whole tissue sections. IDH2 R172M and IDH2 R172K cases showed negative immunostaining for MsMab-2.

DISCUSSION

Our study demonstrated that IDH1 R132L could be precisely identified using FFPE samples with the rat monoclonal antibody MsMab-2. The number of antibodies specific to mutant IDH1/2 and available for FFPE specimens is limited^{7,12} and MsMab-2 was the first antibody to be developed that can detect IDH1 R132L specifically using tissue sections from FFPE samples. We therefore consider immunohistochemical analysis to be a useful method of detecting IDH1 R132L and diagnosing ICC. Though sequencing analysis is effective at detecting somatic mutations, immunohistochemical analysis is more convenient, especially for pathologists. Additionally, it is advantageous when PCR proves difficult because of high DNA fragmentation.

We previously reported MsMab-2 immunoreactivity against IDH2 R172M using western blot analysis,⁸ but our present findings revealed no immunoreactivity in the immunohistochemical analysis of an IDH2 R172M case using an FFPE sample. While antibodies may differ in immunoreactivity between immunohistochemical and western blot analysis,¹³ we think that further analyses are necessary to confirm MsMab-2 immunoreactivity because of the small number of IDH2 R172M cases in this study.

An *IDH* mutation was first discovered in colorectal cancers during the consensus coding sequence project.¹⁴ Since then, genome studies have identified somatic *IDH* mutations in several cancers, including glioma,⁵ leukemia,¹⁵ and intrahepatic cholangiocarcinoma.³ Most *IDH1* mutations occur at arginine 132; this is thought to be a functional domain, so the mutation presumably results in oncogenic enzymatic activity.¹⁶ Additionally, a molecular study showed that mutant IDH1 can prevent

histone demethylation through 2-hydroxyglutarate production from α -ketoglutarate.¹⁷ This epigenetic dysregulation and the following expression profile alteration are considered to promote apoptosis resistance, migration, and invasion.¹⁸

Out of six *IDH1* R132 variants, R132C, R132H, R132G, R132L, and R132S have been identified in previous studies, and R132C and R132H are the most common.¹⁹ IDH1 R132 mutant expression is known to vary by tumor type, but the clinicopathological features of each IDH1 R132 variant remains unclear. Regarding cholangiocarcinoma specifically, no studies have identified differences in clinicopathological characteristics between IDH1 R132 variants.¹⁹ Our previous study identified no statistically significant differences in the characteristics of R132C, R132G, and R132L, though the sample size was limited (data not shown).

IDH1 R132L is not a common IDH1 mutation according to the Catalogue of Somatic Mutations in Cancer (COSMIC) (<http://cancer.sanger.ac.uk/cosmic>). However, a study of *IDH* mutations in mesenchymal tumors found that *IDH1* R132L was more common in some types of tumors than expected.⁶ Moreover, we previously showed¹¹ that *IDH1* R132L was more frequent in ICC than in previous studies.^{3,9} Because ICC ratios in liver tumors differ between countries,²⁰ this suggests that *IDH1* R132L frequencies in ICC differ between regions, races, or as yet undetermined risk factors.

In conclusion, we demonstrate the precise immunohistochemical detection of IDH1 R132L in ICC FFPE samples using the rat monoclonal antibody MsMab-2. We believe that this method will be useful in the detection or classification of a variety of tumors, including ICC, in routine diagnosis. In addition, we anticipate that other antibodies specific for the IDH1 R132 mutant, which is more frequent in ICC (i.e., IDH1 R132C), will be available in the future.

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DISCLOSURE STATEMENT

None declared.

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Table 1 Genetic *IDH* status based on MsMab-2 immunoreactivity

<i>IDH</i> status	No. of Positive Cases	Percentage
Wild type	0/74	0 %
<i>IDH1</i> -R132L	5/5	100 %
<i>IDH1</i> -R132C	0/11	0 %
<i>IDH1</i> -R132G	0/3	0 %
<i>IDH2</i> -R172M	0/1	0 %
<i>IDH2</i> -R172K	0/1	0 %

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