The Diagnostic Utility of IDH2 R172 Immunohistochemistry in Tall Cell Carcinoma With Reversed Polarity of the Breast

Eiichi Sasaki, MD, PhD,† Akari Iwakoshi, MD, PhD,†‡ Tatsunari Satake, MD,§ Kosei Nakajima, MD, PhD,‖ Mizuho Kobayashi, MD, PhD,¶ Yoshiaki Ando, MD, PhD,# Takashi Koshikawa, MD, PhD,*** Katsuhiro Masago, MD, PhD,* Naoko Honma, MD, PhD,†† Rieko Nishimura, MD, PhD,‡ Yukinari Kato, MD, PhD,‡‡§§ and Shu Ichihara, MD, PhD‡*

Abstract: Tall cell carcinoma with reversed polarity (TCCRP) is a rare histologic type of low-grade breast cancer, consisting of tall columnar cells with reversed nuclear polarity and characterized by frequent *IDH2* mutations. We herein report 3 cases of TCCRP with sequencing analyses of the *IDH2* gene and immunohistochemical examination using monoclonal antibodies (11C8B1) against IDH2 R172. *IDH2* R172 mutations were detected in all 3 resected tumors (R172S in 2 tumors and R172T in 1 tumor), and the presence of these mutations was confirmed by IDH2 R172 immunohistochemistry. Tumor cells of TCCRP showed strong and diffuse staining for the antibody against IDH2 R172. In 1 case, tumor tissue from 2 core needle biopsy samples collected on different days were also immunohistochemically positive for IDH2 R172. These results indicate that IDH2 R172 immunohistochemistry is suitable for the detection of TCCRP in both resection and biopsy samples. In addition, a literature review revealed that R172S and R172T account for 76% of *IDH2* mutations in TCCRP, suggesting that 11C8B1, which reacts with R172S and R172T, was likely most sensitive for *IDH2*-mutated TCCRP among many available antibodies for IDH2 R172. Furthermore, the combination of 2 or more antibodies against IDH2 R172 could be more effective for detecting TCCRP mutation. However, it is important to note that IDH2 R172 immunohistochemistry is not absolute, because *IDH2* wild type is found in a small proportion (10%) of cases, and a few cases of *IDH2*-mutated TCCRP may harbor rare subtypes of R172 that are not covered by available antibodies.

Key Words: tall cell carcinoma with reversed polarity, *IDH2*, R172, breast, 11C8B1

(*Appl Immunohistochem Mol Morphol* 2022;00:000–000)

Tall cell carcinoma with reversed polarity (TCCRP) of the breast is a distinct subtype of triple-negative breast cancer with a favorable prognosis. Histologically, TCCRP is a low-grade carcinoma characterized by tall columnar cells with reversed nuclear polarity, arranged in solid and solid papillary patterns.[1] The tumor was first described by Eusebi et al[2] as a breast tumor resembling the tall cell variant of papillary thyroid carcinoma. Recently, this tumor has been classified as a distinct tumor entity in the fifth edition of the World Health Organization Classification of breast tumors.[3] To date, more than 70 cases of TCCRP have been reported in the literature.

In 2016, Chiang et al[4] found that TCCRP is a breast cancer subtype characterized by frequent *IDH2* mutations. *IDH2* R172 mutations have not been reported in breast cancer cases other than TCCRP, whereas *IDH2* mutations, which did not target the R172 hotspot locus, have only been described in a few cases.[4,5] Therefore, the detection of *IDH2* R172 mutations in breast cancer tissue can be helpful in making a correct diagnosis of TCCRP. An alternative method to the molecular analysis of the *IDH2* gene is immunohistochemistry using mutation-specific antibodies. Many commercially available immunohistochemical monospecific and multispecific antibodies against *IDH2* mutations at codon 172 have been developed,[6–11] but there are no antibodies that cover all the subtypes of *IDH2* R172 mutations. The selection of antibodies can influence the diagnostic utility

Received for publication June 9, 2022; accepted September 15, 2022.

From the *Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital; †Clinical Research Center; ‡Department of Pathology, National Hospital Organization Nagoya Medical Center; §Department of Pathology, Nagoya Ekisaikai Hospital; ¶Department of Pathology, Nagoya City University West Medical Center; #Ando Breast Clinic, Nagoya; ‖Department of Surgical Pathology, Ichinomiya Municipal Hospital; **Department of Medical Technology, Shubun University Faculty of Medical Sciences, Ichinomiya; ††Department of Pathology, Toho University Faculty of Medicine, Tokyo; Departments of ‡‡Antibody Drug Development; and §§Molecular Pharmacology, Tohoku University Graduate School of Medicine, Sendai, Japan.

Supported by Nagoya Medical Center and by the Aichi Cancer Research Foundation.

E.S. and S.I. conceived and designed the study. E.S., A.I., T.S., K.N., M.K., Y.A., T.K., R.N., and S.I. contributed to the materials and patients. E.S., R.N., and S.I. provided a pathology review. E.S., K.M., and S.I. analyzed and interpreted the data. E.S. and S.I. wrote the paper. E.S. generated tables and figures. K.M., N.H., R.N., and Y.K. reviewed and edited the paper.

The authors declare no conflict of interest.

Reprints: Eiichi Sasaki, MD, PhD, Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya, Aichi 464-8681, Japan (e-mail: sasakia1es@gmail.com).

of IDH2 R172 immunohistochemistry. However, this point has not been discussed in previous studies of TCCRP.

In this study, we report 3 cases of TCCRP and review the relevant literature to clarify the best immunohistochemical panel for the diagnosis of TCCRP. Furthermore, we assessed the programmed death-ligand 1 (PD-L1) expression in TCCRP to consider the possibility of immune checkpoint inhibitor (ICI) treatment for this tumor.

MATERIALS AND METHODS

Patients and Histologic Evaluation

We selected 3 cases of TCCRP of the breast from the consultation archive of one of the authors (S.I.). Resected specimens from all the 3 cases were evaluated. For 1 case, 2 core needle biopsy samples collected on different days were also evaluated. All diagnoses were confirmed by 3 pathologists (E.S., R.N., and S.I.). TCCRP was diagnosed according to the most recent version of the World Health Organization classification.[3] We also selected and retrieved 6 cases of non-TCCRP breast carcinoma to compare the IDH2 R172 expression with its expression in TCCRP. These 6 tumors included 5 triple-negative breast carcinomas (2 of secretory carcinoma, 1 of carcinoma with apocrine differentiation, 1 of metaplastic carcinoma, and 1 of invasive carcinoma of no special type) and 1 hormone receptor-positive invasive lobular carcinoma. All tissues were fixed in 10% formalin and embedded in paraffin. All procedures used in this research were approved by the Ethical Committee of Nagoya Medical Center (No. 2006-15). This study was conducted in accordance with the Declaration of Helsinki.

Immunohistochemistry

Immunohistochemistry was performed on 4-μm-thick formalin-fixed paraffin-embedded (FFPE) sections. The following antibodies were used: ER [SP1, ready-to-use (RTU); Roche], PgR (E12, RTU; Roche), HER2 (4B5, RTU; Roche), ki67 (MIB-1, 1:50; Dako), p63 (4A4, RTU; Roche), CK5/6 (D5/16B4, RTU; Roche), S100 (Polyclonal Rabbit, RTU; Dako), CD31 (JC70A, RTU; Dako), GATA3 (HG3-31, 1:50), SOX10 (E6B6I, 1:50; Cell Signaling), PAX8 (Polyclonal Rabbit, 1:1500; Proteintech), Calretinin (DAK-Calret1, 1:100; Dako), mammaglobin (304-1A5, RTU; Dako), GCDFP15 (EP95, RTU; BioGenex), MUC4 (8G7, 1:200; Santa Cruz), BRAF V600E (VE1, RTU; Roche), epidermal growth factor receptor (EGFR) (31G7, RTU; Nichirei Biosciences), pan-TRK (EPR17341, RTU; Roche), TTF-1 (8G7G3/1, 1:200; Dako), PD-L1 (SP142, RTU; Roche, and 22C3, RTU; Dako), and IDH2 R172 (11C8B1, 1:2000; NewEast Biosciences). PD-L1 status was assessed with clone SP142 using the OptiView DAB IHC Detection Kit (Ventana Medical Systems) and with clone 22C3 using the Dako pharmDx Kit (Dako, Agilent Technologies). SP142-stained slides were scored for PD-L1 immune cell (IC) positivity,[12] whereas 22C3-stained slides were read for the PD-L1 combined positivity score (CPS).[13]

Mutation Analysis

DNA was extracted from the tumor areas of each unstained FFPE section with reference to hematoxylin and eosin–stained specimens. Sample DNA was amplified with the following primer set encompassing the *IDH2* R172 hotspot locus: forward primer, TCAAGCTGAAGAAGATGTGGAA; and reverse primer, CAAAGTCTGTGGCCTTGTACTG. The products were directly sequenced using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

RESULTS

Clinical Findings

The patients were 3 women, with an average age of 57 years (44, 55, and 71 y, respectively) (Table 1). All the 3 patients were Japanese, an ethnic group in which TCCRP has not been reported thus far. The average tumor size at excision was 9 mm (4, 9, and 14 mm, respectively). The laterality was left (n = 2) and right (n = 1). For 1 patient, follow-up observation before excision was performed for 3 years, as it was diagnosed as a benign lesion, likely intraductal papilloma, based on the pathologic examination of a biopsy specimen obtained at a previous institute (Case 3). In Case 3, the tumor size increased slightly in the follow-up observation period. All patients underwent surgical excision with sentinel lymph node resection. In all the 3 cases, the surgical margin was free and no sentinel lymph node metastasis was seen. The 3 patients were followed for 19, 15, and 8 months after resection, respectively. No patients developed local recurrence or metastasis.

Pathologic and Immunohistochemical Findings

All the 3 tumors showed similar pathologic features. These tumors were composed of multilobulated nests with solid and solid papillary structure separated by dense fibrous stroma (Fig. 1A). The tumor cells were tall and columnar, with abundant eosinophilic cytoplasm and round to ovoid nuclei. The presence of nuclei at the apical sides, a striking histologic feature of TCCRP, was found in the periphery of the tumor nests (Fig. 1B). Follicular-like structures containing eosinophilic material were seen in all the 3 cases, at least focally (Fig. 1C). Mitotic figures, if present, were rare (< 1 per 10 high power fields). Necrosis and vascular invasion were absent

TABLE 1. Clinicopathologic Features of Tall Cell Carcinoma With Reversed Polarity

Case No.	Age (y)	Sex	Laterality	Size (mm)	Presentation	Lymph Nodes	Treatment	Follow-up
1	55	F	Left	4	Palpable nodule	SN negative	Wide excision	NED (19 mo)
2	44	F	Right	9	Palpable nodule	SN negative	Wide excision	NED (15 mo)
3	71	F	Left	14	Palpable nodule present 3y	SN negative	Wide excision	NED (8 mo)

F indicates female; NED, no evidence of disease; SN, sentinel lymph node.

in all the 3 cases. Tumor-infiltrating lymphocytes were sparse, and the percentage of stromal tumor-infiltrating lymphocytes was 0% to 1% in all the 3 tumors.

In Case 3, 2 specimens biopsied on 2 different days (3 y and 1 mo before surgical excision, respectively) showed identical histologic findings to the surgical specimen. Therefore, the former tumor sampled 3 years previously was reclassified as TCCRP, although the original diagnosis was benign papilloma.

The key results from the immunohistochemical analysis are summarized in Table 2. One tumor had a triple-negative phenotype, and the remaining 2 tumors showed focal hormone receptor expression. The Ki67 proliferation index was low (up to 3%). All the 3 tumors were positive for CK5/6 (2 cases, diffuse; 1 case, focal), calretinin (diffuse), GCDFP15 (focal), and EGFR (focal, weak) (Figs. 1D–F). Occasional positivity was seen for GATA3 (2/3, focal), mammaglobin (2/3, focal), and S100 (2/3, focal and weak), whereas all the 3 tumors were negative for TTF-1, PAX8, SOX10, and MUC4. PD-L1 SP142 IC and 22C3 combined positivity score were <1% in all the 3 tumors (Fig. 1G). CD31 staining highlighted numerous capillaries in the basal lamina (Fig. 1H). Tumor islands surrounded by a layer of p63-positive myoepithelial cells were not found in any tumor areas. Flat epithelial atypia, atypical ductal hyperplasia, ductal carcinoma in situ, and microglandular adenosis, all of which can be precursors of invasive breast cancer, were not found in the background breast tissue.

Genetic Findings by Sanger Sequencing and Immunohistochemistry Using a Mutation-specific Antibody

IDH2 mutations were detected in all the 3 resected tumors by Sanger sequencing. Two types of *IDH2* mutation,

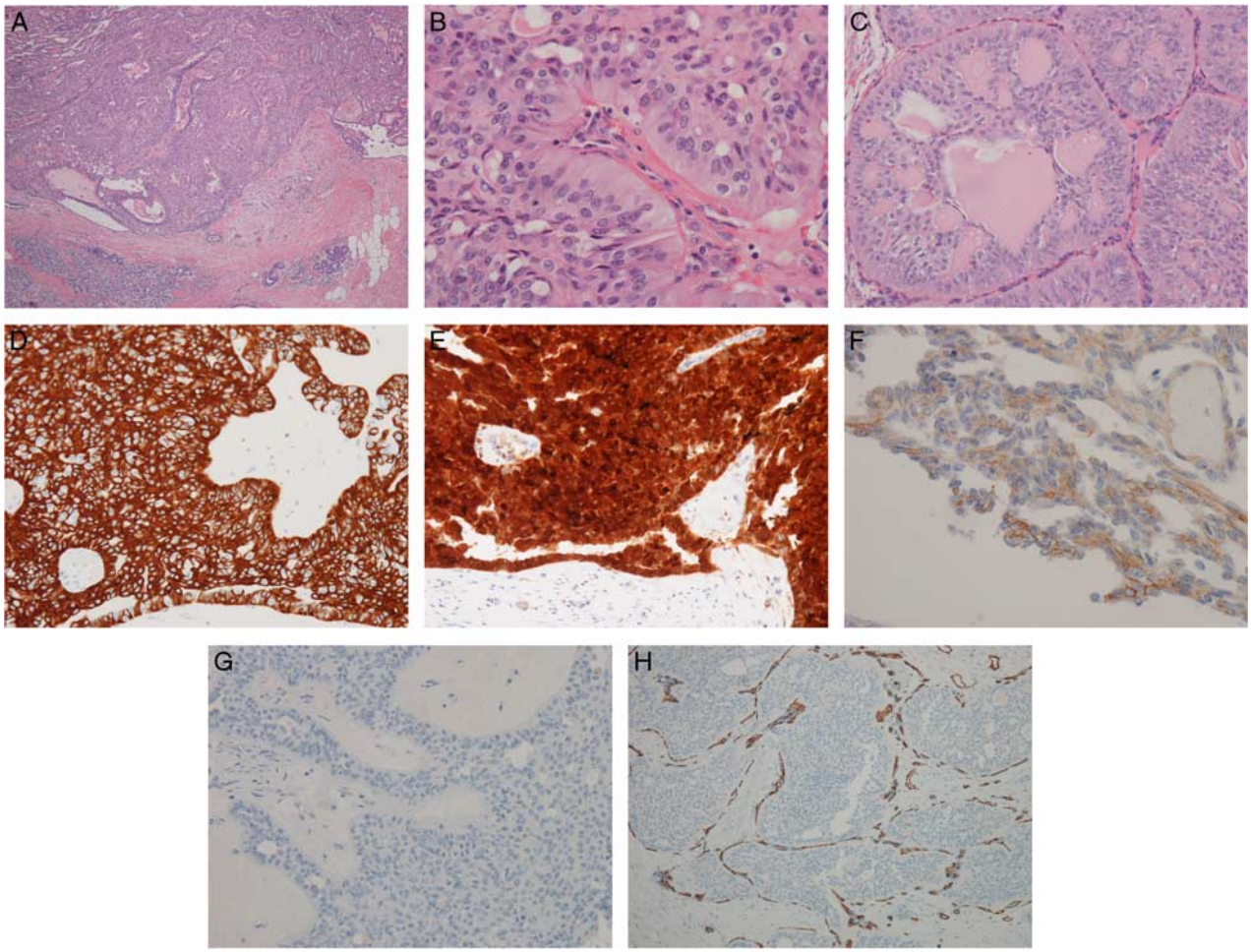

FIGURE 1. The histologic and immunohistochemical findings of tall cell carcinoma with reversed polarity. A, At low-power magnification, the tumor shows a pushing margin. B, Tumor cells have abundant eosinophilic cytoplasm and reverse polarity, characterized by apical localization of the nuclei. C, Follicle-like structures with colloid-like secretion are found. D, Tumor cells are positive for CK5/6. E, Diffuse and strong positivity for calretinin is seen in both the nuclei and cytoplasm of tumor cells. F, Membranous staining of epidermal growth factor receptor is focally present. G, Tumor cells and immune cells are negative for 22C3, and the programmed death-ligand 1 22C3 combined positive score is <1%. H, CD31 highlights the capillary network along to the basal lamina.

TABLE 2. Immunohistochemical Findings of Tall Cell Carcinoma With Reversed Polarity

Case No.	ER %	PgR %	HER2	Ki67%	CK5/6	Calretinin	EGFR	GCDFP15	PAX8	SOX10	MUC4	PD-L1 22C3 CPS	PD-L1 SP142 IC	IDH2 R172	BRAF V600E	Pan-TRK
1	10	2	0	2	+, diffuse	+, diffuse	+, focal	+, focal	—	—	—	< 1%	< 1%	+, diffuse	—	—
2	2	5	1+	3	+, diffuse	+, diffuse	+, focal	+, focal	—	—	—	< 1%	< 1%	+, diffuse	—	—
3	0	0	0	2	+, focal	+, diffuse	+, focal	+, focal	—	—	—	< 1%	< 1%	+, diffuse*	—	—

*In Case 3, diffuse positive reactivity for IDH2 R172 was observed in 2 core needle biopsy samples collected on different days and an excision sample.
CPS, combined positive score; EGFR, epidermal growth factor receptor IC, immune cells.

R172S and R172T, were found in 2 cases (Cases 1 and 2) and 1 case (Case 3), respectively (Fig. 2A). To validate the presence of these mutations and the distribution of mutated tumor cells, we performed an immunohistochemical analysis using monoclonal antibodies (11C8B1) against the *IDH2* R172 mutation. All 3 tumors, irrespective of the mutation types of R172S or R172T, were positive for the antibodies, with diffuse and strong positivity for tumor cells (Figs. 2B, C). In Case 3, an *IDH2* R172T mutation that was identical to the resected sample was detected in a sample biopsied 1 month before excision. In another sample biopsied 3 years previously, PCR products were not amplified because of the low quality of extracted DNA and the mutation status could not be analyzed. However, diffuse and strong positivity for IDH2 R172 was observed in tumor cells in both the 2 core needle biopsy samples, irrespective of the DNA quality.

In addition, we performed an immunohistochemical analysis using a BRAF V600E mutation-specific antibody, because the mutations are most common in papillary thyroid carcinoma, a tumor with similar histologic features to TCCRP. All the 3 tumors were immunohistochemically negative for BRAF V600E mutation-specific antibody, which is highly specific and sensitive for the V600E mutation.[14] Furthermore, all the 3 tumors were negative for pan-TRK, a surrogate marker for the identification of *NTRK* fusion.[15]

Immunohistochemically, IDH2 R172 was negative in all 6 non-TCCRP tumors.

DISCUSSION

Mutations in the *IDH2* gene result in the simultaneous loss of normal catalytic activity, the production of a-ketoglutarate (α-KG), and gain of a new function, the production of 2-hydroxyglutarate (2-HG), which causes altered stem cell differentiation and eventual tumorigenesis.[16] *IDH2* mutation has been reported in relatively restricted tumor types, including glioma, acute myeloid leukemia, cartilaginous tumor, cholangiocarcinoma, and sinonasal undifferentiated carcinoma and is assumed to play an important role in the carcinogenesis of these tumors.[16] In this study, we conducted a mutational analysis of 3 cases of TCCRP and found that all the 3 tumors harbored *IDH2* R172 mutations. The *IDH2* mutations were also confirmed by immunohistochemistry using monoclonal antibodies to the *IDH2* R172 mutation and almost all tumor cells of these cases were positive for the antibodies. In addition, in 1 case, the results of the immunohistochemistry using IDH2 R172 antibodies were in agreement among 3 matched samples (2 core needle biopsy samples and an excision sample) collected on different days during the clinical course of 3 years. These findings suggest that the mutation is an early oncogenic event during the tumorigenesis of TCCRP.

11C8B1, which was used in this study, was originally designated as an antibody against IDH2 R172S, but our study and a previous study[17] have shown that this antibody is positive not only for TCCRP with R172S mutations but also the tumors with R172T mutations. No reaction with other variants of R172 (R172K/G/W/M/I)

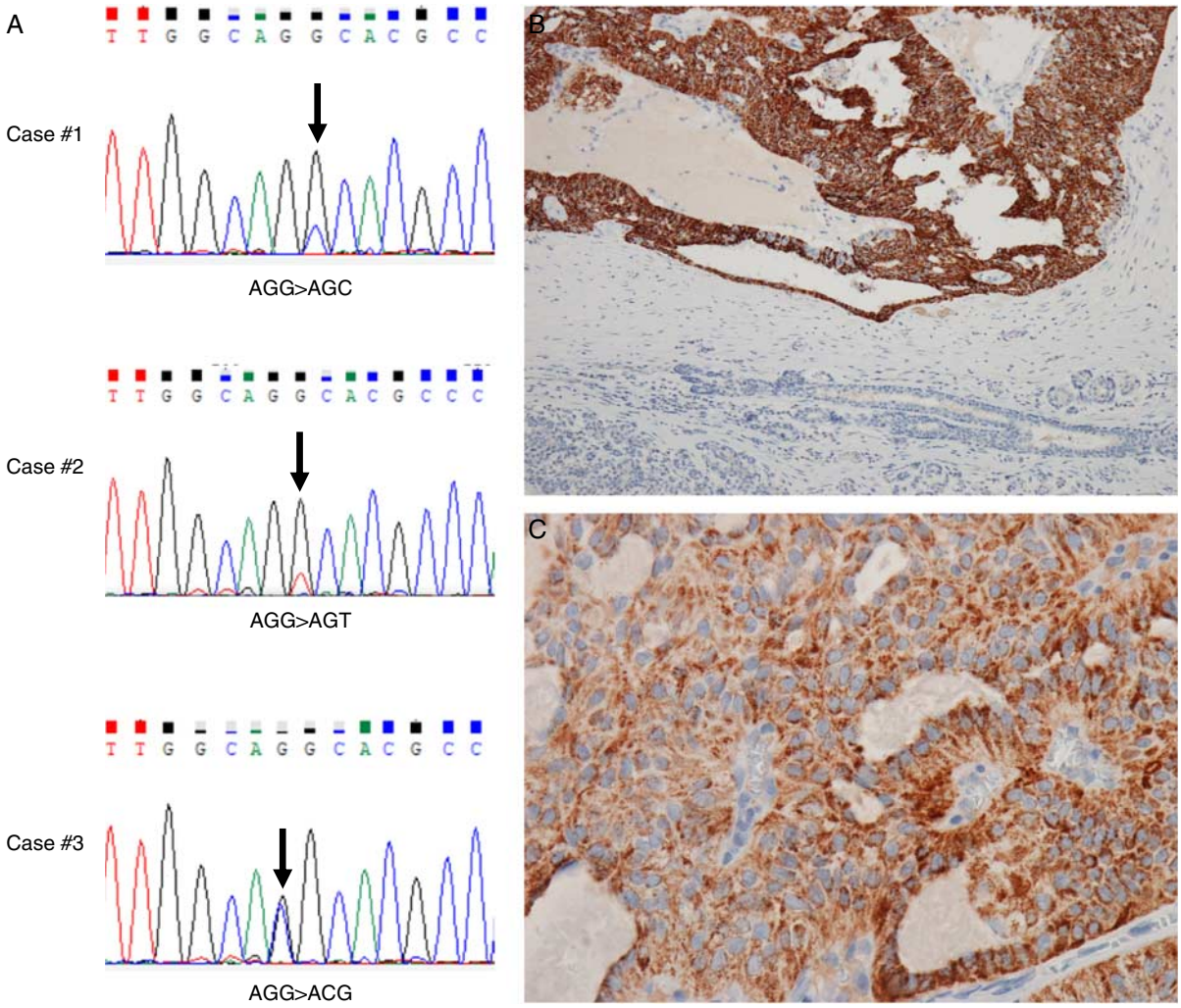

FIGURE 2. Detection of *IDH2* R172 mutations by Sanger sequencing and immunohistochemistry. A, Sanger sequencing revealed *IDH2* R172 mutations in all 3 cases: c.516G > C (p.R172S) in Case 1, c.516G > T (p.R172S) in Case 2, and c.515G > C (p.R172T) in Case 3. B, The tumor cells of Case 2 show strong and diffuse immunostaining for 11C8B1, whereas normal breast tissue at the lower side of the picture is negative for this marker. C, At high-power magnification, the tumor cells of Case 3 are positive in the cytoplasm, with a granular pattern.

was found in previous studies that examined 11C8B1 immunohistochemistry in various tumor types with *IDH2* mutations.[7,17]

The distribution of *IDH2* mutation types in a total of 60 TCCRP cases with sequencing analyses for the *IDH2* gene reported thus far are summarized in Table 3.[4,17–26] *IDH2* mutation was detected in 90% of the cases (54/60), and the mutational regions were limited to codon 172. Five subtypes of *IDH2* R172 mutation [R172S, R172T, R172G, R172W, and R172I (in descending order)] have been reported. No antibodies cover all 5 subtypes of *IDH2* R172 mutations reported in TCCRP, although there are many immunohistochemical antibodies against IDH2 R172 available on FFPE sections. Importantly, 11C8B1 seems to have the highest detection rate for *IDH2*-mutated TCCRP; R172S and R172T, which are detectable by 11C8B1, account for 76% (41/54) of the R172 mutations in these tumors (Table 3). An alternative marker is MsMab1, a multispecific antibody that reacts with R172S, R172G, and R172M, but shows negativity for R172T, R172K, R172W, and R172I.[27] The estimated rate of MsMab1 positivity in *IDH2*-mutated TCCRP is 63% (34/54). IDH2 R172W, with which neither 11C8B1 nor MsMab1 react, can be detected by a monospecific antibody, WMab1.[9] To the best of our knowledge, no available antibody against IDH2 R172I has been developed. Therefore, the combination of 11C8B1 and MsMab1, or plus WMab1, could be expected to detect almost all *IDH2*-mutated TCCRPs [94% (51/54) or 98% (53/54), respectively].

Our study, like a previous study,[17] showed that IDH2 R172 immunohistochemistry of core needle biopsy specimens of TCCRP is feasible. The evaluation for IDH2 R172 staining is unlikely affected by the site sampled via biopsy or fine needle aspiration, because of the homogeneous and

TABLE 3. Distribution of *IDH2* Mutations in Previously Reported Cases of Tall Cell Carcinoma With Reversed Polarity With Genetic Analyses

References	n	Methodology	R172S*	R172T*	R172G*	R172W†	R172I	Wild Type
Chiang et al[4]	13	NGS	3	4	3	—	—	3
Bhargava et al[18]	3	NGS	1	1		—	—	1
Lozada et al[19]	6	Sanger sequencing	3	2	1	—	—	—
Zhong et al[20]	7	NGS	4	2	—	1	—	—
Haefliger et al[21]	1	NGS	—	—	1	—	—	—
Alsadoun et al[22]	9	NGS	3	1	3	—	—	2
Pareja et al[17]	14	Sanger sequencing	7	6	—	—	1	—
Wei et al[23]	2	NGS	1	—	—	1	—	—
Jassim et al[24]	1	Sanger sequencing	—	—	1	—	—	—
Zhang et al[25,26]	1	NGS	—	—	1‡	—	—	—
This study	3	Sanger sequencing	2	1	—	—	—	—
Total	60	—	24	17	10	2	1	6

NGS indicates next generation sequencing.
*Among multispecific antibodies against *IDH2* mutations at codon 172, 11C8B1 reacts with R172S and R172T, whereas MsMab1 reacts with R172S and R172G.
†WMab1, monospecific antibody reacts with R172W.
‡The mutation was originally reported as *IDH2* R120G, a novel mutation, rather than *IDH2* R172,[25] but we realized that the mutation was actually R172G.[26]

robust staining. Notably, in 1 case, tumor tissue from an archived core needle biopsy sample was inadequate for a genetic analysis because of low DNA quality but was immunohistochemically positive for IDH2 R172. The superiority of immunohistochemistry to genetic sequencing for the detection of mutation-specific antibodies may be remarkable when archived tumor tissue samples with decreasing DNA quality are used, or when using specimens with a small number of tumor cells or low-tumor purity are used.[28]

Pareja et al[17] reported that none of 226 triple-negative breast cancers or 34 benign or low-grade histologic mimics of TCCRP, including 13 intraductal papillomas, 16 solid papillary carcinomas, and 5 encapsulated papillary carcinomas, expressed IDH2 R172 by immunohistochemistry using monoclonal antibodies (11C8B1). Although our study had a small sample size, it included histologic types such as secretory carcinoma, for which the IDH2 R172 immunohistochemistry has not been examined (or described) in previous studies. Our study further confirmed the specificity of IDH2 R172 immunohistochemistry in TCCRP among various histologic types of breast tumors.

Our TCCRPs were invariably positive for calretinin. Calretinin seems to be a useful marker for the differential diagnosis of TCCRP among low-grade breast lesions, because the expression of calretinin has been reported to be rare in low-grade breast cancer.[29,30] Contrary to low-grade cancer, high-grade breast cancers are frequently positive for calretinin. Interestingly, among high-grade breast cancers, the expression of calretinin is more frequently seen in association with the expression of CK5/6.[30,31] TCCRP is also positive for CK5/6. In addition, our cases of TCCRP showed positivity—albeit weak positivity—for EGFR. Which intrinsic subtype of breast cancer TCCRP falls into is unclear. A previous study using RNA sequencing showed that 5 of the 9 tumors were categorized as luminal-A type and 4 others were classified as basal type.[22] However, these shared immunohistochemical features of TCCRP and TNBC with a basal-like phenotype (CK5/6 and EGFR positive) may facilitate the understanding of TCCRP, in terms of the tumor origin and differentiation.

TCCRP is assumed to be an invasive cancer because of the absence of myoepithelial cells around tumor nests and the presence of metastatic lesions in a small subset of cases, but the precursor lesion of TCCRP remains unknown. Two of 3 tumors in our study were small (< 10 mm in size), and, to our knowledge, the tumor size of Case 1 (4 mm) is the smallest ever reported,[3] suggesting that these tumors may represent lesions at an early stage of invasive cancer. However, in situ carcinoma components surrounded by a layer of myoepithelial cells were not found in any tumor areas of our 3 TCCRPs, although a preexisting precursor lesion may be completely overtaken by carcinoma with progression. Therefore, the multistep progression model of invasive breast cancer via ductal carcinoma in situ seems not to be applicable for TCCRP. These histologic findings of TCCRP may reflect the biological nature as basal type rather than luminal type.

Follicular-like structures with colloid-like secretion are frequently seen in TCCRP cases, as shown in Figure 1C. Despite its morphological characteristics, which resemble thyroid cancer, variable staining for GCDFP15, mammaglobin, and GATA3 combined with negativity for TTF-1 and PAX8 suggest a tumor of breast—not thyroid—origin.[32,33] Colloid-like secretions of TCCRP also may mimic the extracellular secretions of a secretory carcinoma, a different tumor entity from TCCRP, which is frequently associated with *ETV6-NTRK3* fusion.[34] Therefore, the distinction between TCCRP and secretory carcinoma may be difficult in small biopsy specimens, and an immunohistochemical analysis may be needed to make a correct diagnosis. Tumor cells of secretory carcinoma are frequently positive for SOX10, MUC4, and pan-TRK,[15,35,36] while our study showed that TCCRPs were negative for these 3 markers.

TCCRP is a low-grade breast cancer and has an indolent clinical course, without disease recurrence in the

majority of patients. In this study, no local recurrence or metastasis were found in any of the 3 TCCRP cases, including a case that was followed for 3 years follow-up period before resection. However, an exceptional case with distant metastasis of the bone was reported.[37] Recently, IDH1/2 inhibitors were shown to be effective against *IDH2*-mutated cancer.[38] When a TCCRP shows aggressive clinical behavior, IDH1/2 inhibitors may be a therapeutic option. In contrast, ICIs might be rarely indicated for the treatment of TCCRP, a tumor that usually exhibits a triple-negative phenotype. Our study showed that the PD-L1 status in TCCRP was lower than the positive cutoff value for ICI therapy, although the efficacy of the combination ICIs with chemotherapy for advanced triple-negative breast cancer has been shown in a subgroup of patients with PD-L1-positive tumors.[12,13]

To the best of our knowledge, there is no literature regarding the cytologic findings in TCCRP. Furthermore, none of our 3 cases had any cytology specimens obtained via fine-needle aspiration or touch imprint. Further studies will be needed to clarify whether or not the diagnostic utility of R172 immunohistochemistry in TCCRP applies not only to FFPE sections but also to cytology specimens among breast tumors.

In summary, we described 3 cases of TCCRP with an immunohistochemical analysis of *IDH2* R172 hotspot mutations. Immunohistochemistry using antibodies against IDH2 R172 is a sensitive and specific test for the identification of TCCRP harboring *IDH2* R172 mutations. However, there are no antibodies that cover all subtypes of *IDH2* R172 mutations reported in TCCRP, including multispecific antibodies such as 11C8B1 and MsMab1. Therefore, the combination of 2 or more antibodies against R172 could be more effective for the diagnosis of TCCRP. In addition, *IDH2* R172 mutations have not been detected in a minority of cases of TCCRP (~10%) (Table 3). In such cases, a detailed histologic evaluation combined with an immunohistochemical analysis using other markers, including calretinin and CK5/6 can help make a correct diagnosis of TCCRP.

ACKNOWLEDGMENTS

The authors thank Yukiko Takahashi and Kyoko Kato for secretarial assistance.

REFERENCES

1. Foschini MP, Asioli S, Foreid S, et al. Solid Papillary Breast Carcinomas Resembling the Tall Cell Variant of Papillary Thyroid Neoplasms: A Unique Invasive Tumor With Indolent Behavior. *Am J Surg Pathol* . 2017;41:887–895.
2. Eusebi V, Damiani S, Ellis IO, et al. Breast tumor resembling the tall cell variant of papillary thyroid carcinoma: report of 5 cases. *Am J Surg Pathol*. 2003;27:1114–1118.
3. Yang W, Bu H, Foschini M, et al. Tall cell carcinoma with reversed polarity. In: Allison K, Brogi E, Ellis I, Fox S, Morris E, Sahin A, Salgado R, Sapino A, Sasano H, Schnitt S, Sotiriou C, van Diest P, eds. *Breast Tumours*. 2019 International Agency for Research on Cancer; 153–154.
4. Chiang S, Weigelt B, Wen HC, et al. IDH2 mutations define a unique subtype of breast cancer with altered nuclear polarity. *Cancer Res*. 2016;76:7118–7129.
5. Razavi P, Chang MT, Xu G, et al. The genomic landscape of endocrine-resistant advanced breast cancers. *Cancer Cell*. 2018;34:427–438.e426.
6. Kato Y. Specific monoclonal antibodies against IDH1/2 mutations as diagnostic tools for gliomas. *Brain Tumor Pathol*. 2015;32:3–11.
7. Dogan S, Frosina D, Fayad M, et al. The role of a monoclonal antibody 11C8B1 as a diagnostic marker of IDH2-mutated sinonasal undifferentiated carcinoma. *Mod Pathol*. 2019;32:205–215.
8. Dogan S, Frosina D, Geronimo JA, et al. Molecular epidemiology of IDH2 hotspot mutations in cancer and immunohistochemical detection of R172K, R172G, and R172M variants. *Hum Pathol*. 2020;106:45–53.
9. Kato Y, Kaneko MK. Generation of a novel monoclonal antibody WMab-1 specific for IDH2-R172W mutation. *Biochem Biophys Res Commun*. 2013;433:374–378.
10. Liu X, Ogasawara S, Kaneko MK, et al. A novel monoclonal antibody SMab-2 recognizes endogenous IDH2-R172S of chondrosarcoma. *Biochem Biophys Res Commun*. 2015;459:636–642.
11. Kaneko MK, Morita S, Tsujimoto Y, et al. Establishment of novel monoclonal antibodies KMab-1 and MMab-1 specific for IDH2 mutations. *Biochem Biophys Res Commun*. 2013;432:40–45.
12. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-Paclitaxel in advanced triple-negative breast cancer. *N Engl J Med*. 2018;379:2108–2121.
13. Cortes J, Cescon DW, Rugo HS, et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet*. 2020;396:1817–1828.
14. Seto K, Haneda M, Masago K, et al. Negative reactions of BRAF mutation-specific immunohistochemistry to non-V600E mutations of BRAF. *Pathol Int*. 2020;70:253–261.
15. Harrison BT, Fowler E, Krings G, et al. Pan-TRK immunohistochemistry: a useful diagnostic adjunct for secretory carcinoma of the breast. *Am J Surg Pathol*. 2019;43:1693–1700.
16. Yang H, Ye D, Guan KL, et al. IDH1 and IDH2 mutations in tumorigenesis: mechanistic insights and clinical perspectives. *Clin Cancer Res*. 2012;18:5562–5571.
17. Pareja F, da Silva EM, Frosina D, et al. Immunohistochemical analysis of IDH2 R172 hotspot mutations in breast papillary neoplasms: applications in the diagnosis of tall cell carcinoma with reverse polarity. *Mod Pathol*. 2020;33:1056–1064.
18. Bhargava R, Florea AV, Pelmus M, et al. Breast Tumor Resembling Tall Cell Variant of Papillary Thyroid Carcinoma: A Solid Papillary Neoplasm With Characteristic Immunohistochemical Profile and Few Recurrent Mutations. *Am J Clin Pathol*. 2017;147:399–410.
19. Lozada JR, Basili T, Pareja F, et al. Solid papillary breast carcinomas resembling the tall cell variant of papillary thyroid neoplasms (solid papillary carcinomas with reverse polarity) harbour recurrent mutations affecting IDH2 and PIK3CA: a validation cohort. *Histopathology*. 2018;73:339–344.
20. Zhong E, Scognamiglio T, D'Alfonso T, et al. Breast tumor resembling the tall cell variant of papillary thyroid carcinoma: molecular characterization by next-generation sequencing and histopathological comparison with tall cell papillary carcinoma of thyroid. *Int J Surg Pathol*. 2019;27:134–141.
21. Haefliger S, Muenst S, Went P, et al. Tall cell carcinoma of the breast with reversed polarity (TCCRP) with mutations in the IDH2 and PIK3CA genes: a case report. *Molecular biology reports*. 2020;47:4917–4921.
22. Alsadoun N, MacGrogan G, Truntzer C, et al. Solid papillary carcinoma with reverse polarity of the breast harbors specific morphologic, immunohistochemical and molecular profile in comparison with other benign or malignant papillary lesions of the breast: a comparative study of 9 additional cases. *Mod Pathol*. 2018;31:1367–1380.
23. Wei Y, Ding L, Song X, et al. Tall cell carcinoma with reversed polarity: case report with gene sequencing and literature review. *Gland Surg*. 2021;10:3147–3154.
24. Jassim M, Premalata CS, Okaly G, et al. Tall cell carcinoma with reverse polarity of breast: report of a case with unique morphologic and molecular features. *Turk Patoloji Derg*. 2021;37:183–188.
25. Zhang X, Wu H, Wang Z, et al. Tall cell carcinoma of the breast with reverse polarity: case report with gene sequencing and literature review. *Gland Surg*. 2021;10:837–843.

26. Sasaki E, Masago K. Codon 172 in the IDH2 gene is a mutational hotspot in tall cell carcinoma with reversed polarity of the breast. *Gland Surg*. 2022;11:1127–1129.
27. Liu X, Kato Y, Kaneko MK, et al. Isocitrate dehydrogenase 2 mutation is a frequent event in osteosarcoma detected by a multi-specific monoclonal antibody MsMab-1. *Cancer medicine*. 2013;2: 803–814.
28. Sasaki E, Masago K, Fujita S, et al. AKT1 mutations in peripheral bronchiolar papilloma: glandular papilloma and mixed squamous cell and glandular papilloma is distinct from bronchiolar adenoma. *Am J Surg Pathol*. 2021;45:119–126.
29. Powell G, Roche H, Roche WR. Expression of calretinin by breast carcinoma and the potential for misdiagnosis of mesothelioma. *Histopathology*. 2011;59:950–956.
30. Taliano RJ, Lu S, Singh K, et al. Calretinin expression in high-grade invasive ductal carcinoma of the breast is associated with basal-like subtype and unfavorable prognosis. *Hum Pathol*. 2013;44: 2743–2750.
31. Micello D, Bossi A, Marando A, et al. Expression of calretinin in high-grade hormone receptor-negative invasive breast carcinomas: correlation with histological and molecular subtypes. *Virchows Arch*. 2017;471:13–21.
32. Krings G, Nystrom M, Mehdi I, et al. Diagnostic utility and sensitivities of GATA3 antibodies in triple-negative breast cancer. *Hum Pathol*. 2014;45:2225–2232.
33. Sasaki E, Tsunoda N, Hatanaka Y, et al. Breast-specific expression of MGB1/mammaglobin: an examination of 480 tumors from various organs and clinicopathological analysis of MGB1-positive breast cancers. *Mod Pathol*. 2007;20:208–214.
34. Tognon C, Knezevich SR, Huntsman D, et al. Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. *Cancer Cell*. 2002;2:367–376.
35. Sasaki E, Masago K, Fujita S, et al. Salivary secretory carcinoma harboring a novel ALK fusion: expanding the molecular characterization of carcinomas beyond the ETV6 gene. *Am J Surg Pathol*. 2020;44:962–969.
36. Luk PP, Selinger CI, Eviston TJ, et al. Mammary analogue secretory carcinoma: an evaluation of its clinicopathological and genetic characteristics. *Pathology*. 2015;47:659–666.
37. Cameselle-Teijeiro J, Abdulkader I, Barreiro-Morandeira F, et al. Breast tumor resembling the tall cell variant of papillary thyroid carcinoma: a case report. *Int J Surg Pathol*. 2006;14:79–84.
38. Pirozzi CJ, Yan H. The implications of IDH mutations for cancer development and therapy. *Nat Rev Clin Oncol*. 2021;18:645–661.