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

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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

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

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


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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), BrafV600E (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 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, TCAAGCTGAAAGATGATGAAA; and reverse primer, CAAAGTCTGGCCCTTGTACTG. 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. Clinopathologic 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.

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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,
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 \textit{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 \textit{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 \textit{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.\textsuperscript{14} Furthermore, all the 3 tumors were negative for pan-TRK, a surrogate marker for the identification of NTRK fusion.\textsuperscript{15}

Immunohistochemically, \textit{IDH2} R172 was negative in all 6 non-TCCRP tumors.

**DISCUSSION**

Mutations in the \textit{IDH2} gene result in the simultaneous loss of normal catalytic activity, the production of \(\alpha\)-ketoglutarate (\(\alpha\)-KG), and gain of a new function, the production of 2-hydroxyglutarate (2-HG), which causes altered stem cell differentiation and eventual tumorigenesis.\textsuperscript{16} \textit{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.\textsuperscript{16} In this study, we conducted a mutational analysis of 3 cases of TCCRP and found that all the 3 tumors harbored \textit{IDH2} R172 mutations. The \textit{IDH2} mutations were also confirmed by immunohistochemistry using monoclonal antibodies to the \textit{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\textsuperscript{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)
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
robust staining. Notably, in I 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 al¹⁷ 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 TCCRP’s 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 CK5/6.²⁹,³¹ 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 TCCRP’s, 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,²⁵,³⁵,³⁶ while our study showed that TCCRP’s were negative for these 3 markers.

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

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

<table>
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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. †The mutation was originally reported as IDH2 R120G, a novel mutation, rather than IDH2 R172, but we realized that the mutation was actually R172G.²⁶
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. Re- periodically before resection. However, an exceptional case metastasis were found in any of the 3 TCCRP cases, in-


