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ORIGINAL

Utility of monoclonal PAX8 antibody for distinguishing intrathyroid thymic carcinoma from follicular cell-derived thyroid carcinoma

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Abstract. Follicular cell-derived thyroid carcinomas, including thyroid squamous cell carcinomas (SCCs) and anaplastic carcinomas, are immunoreactive for paired-box gene 8 (PAX8), while non-follicular cell-derived thyroid carcinomas stain negative for the PAX8 antibody. Intrathyroid thymic carcinoma (ITTC) arising from the intrathyroidal ectopic thymus exhibits moderate-to-strong nuclear reactivity for polyclonal PAX8. This is difficult to understand given that PAX8 is not associated with embryonic thymic development. We aimed to determine the diagnostic significance of monoclonal PAX8 antibody in distinguishing ITTCs from follicular cell-derived thyroid carcinomas. Ten ITTCs, 14 poorly differentiated thyroid carcinomas (PDTCs), 14 thyroid SCCs, 7 thymic tissue specimens, 7 thymomas, and 1 thymic carcinoma were analyzed using antibodies against polyclonal and monoclonal PAX8, thyroid transcription factor-1, p63, and CD5. Four ITTCs (40.0%) stained positive for polyclonal PAX8; none stained positive for monoclonal PAX8. All PDTCs and 92.9% of SCCs were immunoreactive for both polyclonal and monoclonal PAX8. All PDTCs, 46.2% of SCCs, and none of the ITTCs were immunoreactive for thyroid transcription factor-1. Eight ITTCs (80.0%), but none of the PDTCs and SCCs, were immunoreactive for CD5. We are the first to show that ITTCs stain negative for monoclonal PAX8. Monoclonal PAX8 is a more reliable marker than polyclonal PAX8 for determining follicular cell origin. We conclude that monoclonal PAX8 is a useful marker for distinguishing ITTCs from PDTCs and SCCs. Monoclonal PAX8 negativity is additional evidence in support of ITTCs not being follicular cell-derived thyroid carcinomas, but having a thymic origin.

Key words: Immunohistochemistry, Intrathyroid thymic carcinoma, Monoclonal PAX8, Poorly differentiated thyroid carcinoma, Thyroid

INTRATHYROID THYMIC CARCINOMA (ITTC) is a malignant epithelial tumor of the thyroid with thymic epithelial differentiation [1]. It was first reported by Miyauchi *et al.* [2] in 1985 as an intrathyroidal epithelial thymoma. ITTC is an extremely rare entity that has been estimated to account for 0.08–0.15% of primary thyroid malignant tumors [1]. It is believed to arise from the intrathyroidal ectopic thymus, because microscopic and immunohistochemical findings of ITTC and thymic carcinoma are similar [3]. Microscopically, it resembles squamous cell carcinoma (SCC) with a lymphocyte-rich stroma without a follicular or papillary structure [1, 4]. ITTC may occasionally be confused with poorly differentiated thyroid carcinoma (PDTC) or primary thyroid SCC [1]. Immunohistochemically, ITTCs are positive for CD5, tyrosine-protein kinase Kit, and p63, but negative for thyroglobulin and thyroid transcription factor-1 (TTF-1) [1, 4]. Although CD5 is characteristic of ITTCs,

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Abbreviations: ITTC, intrathyroid thymic carcinoma; PAX8, paired-box gene 8; PDTC, poorly differentiated thyroid carcinoma; SCC, squamous cell carcinoma; TTF-1, thyroid transcription factor-1

Table 1 Primary antibodies

Ab	Clone	Manufacturer	AR	Dilution
pPAX8	polyclonal	Proteintech (Chicago, IL, USA)	Heat (pH 6)	1:200
mPAX8	EPR13510	Abcam (Cambridge, UK)	Heat (pH 9)	1:250
TTF-1	8G7G3/1	Dako (Carpinteria, CA, USA)	Heat (pH 6)	1:100
p63	4A4	Nichirei Biosciences (Tokyo, Japan)	Heat (pH 9)	ready to use
CD5	4C7	Leica (Newcastle, UK)	Heat (pH 9)	1:600

Abbreviations: Ab, antibody; AR, antigen retrieval; p, polyclonal; m, monoclonal; PAX8, paired-box gene 8; TTF-1, thyroid transcription factor-1.

some ITTCs stain negative for CD5 antibody [5].

Paired-box gene 8 (PAX8) is a transcription factor that is essential for embryonic thyroid development [6]. Follicular cell-derived thyroid carcinomas, including thyroid SCCs and anaplastic carcinomas, are immunoreactive for PAX8 [7]. Wang *et al.* [8] reported that ITTCs exhibited moderate-to-strong nuclear reactivity for polyclonal PAX8. This is difficult to understand given that PAX8 is not associated with embryonic thymic development. Immunohistochemical analysis using a monoclonal PAX8 antibody may be more reliable and specific than using a polyclonal PAX8 antibody. Herein, we examined the immunoreactivity of both polyclonal and monoclonal PAX8 antibodies in ITTCs and follicular cell-derived thyroid carcinomas. The aim of our study was to determine the diagnostic significance of monoclonal PAX8 antibody in distinguishing ITTCs from follicular cell-derived thyroid carcinomas.

Materials and Methods

Patients and samples

The study protocol was approved by the Institutional Review Board of Kuma Hospital (Hyogo, Japan) (approval number: 20170914-6). We reviewed a pathology report database of 11,587 cases of thyroid carcinoma that were operated on at Kuma Hospital (Hyogo, Japan) between 2003 and 2017. Ten cases of ITTC (0.09%) were extracted. Fourteen PDTCs, 14 primary thyroid SCCs, 7 thymic tissue specimens, 7 thymomas, and 1 primary thymic carcinoma were also extracted. Primary thyroid SCCs included pure SCC and SCC associated with papillary thyroid carcinoma or anaplastic carcinoma, and 11 of these were examined in a previous report [7].

Immunohistochemical staining

Immunohistochemistry was performed using 3.0-μm-thick, formalin-fixed, paraffin-embedded tissue specimens. The primary antibodies used for immunostaining and antigen retrieval methods are listed in Table 1. Staining was performed using the Leica Bondmax system (Leica Microsystems, Wetzlar, Germany) and Bond refine kit (Leica Microsystems, Wetzlar, Germany) according to the manufacturer's recommendations. Cases where >10.0% of the carcinoma cells exhibited moderate-to-strong staining were considered to be immunoreactive [9].

Results

The results of the immunohistochemical analysis are summarized in Table 2. Four ITTCs (40.0%) stained positive for polyclonal PAX8 (Fig. 1A); none of the ITTCs stained positive for monoclonal PAX8 (Fig. 1B). All PDTCs and 92.9% of SCCs were immunoreactive for both polyclonal and monoclonal PAX8. None of the ITTCs stained positive for TTF-1 (Fig. 1C). However, all PDTCs and 42.9% of SCCs were immunoreactive for TTF-1. All ITTCs and SCCs were immunoreactive for p63 (Fig. 1D). Eight ITTCs (80.0%) were immunoreactive for CD5. However, none of the PDTCs and SCCs were immunoreactive for CD5 (Fig. 1E).

Epithelial cells (42.9%) and lymphocytes (57.1%) of the thymus and one thymoma (14.3%) stained positive for polyclonal PAX8, but not monoclonal PAX8 or TTF-1. Epithelial cells at the periphery and in the inner lymph follicles also stained positive for p63.

Discussion

ITTC is a rare neoplasm that arises from the thyroid gland. Histologically and immunophenotypically it resembles thymic carcinoma [1]. It was originally described by Miyauchi *et al.* [2] in 1985 as an intrathyroidal

Table 2 Immunohistochemical staining

Lesion, *n* (%)	Cases	pPAX8	mPAX8	TTF-1	p63	CD5
ITTC	10	4 (40.0)	0 (0.0)	0 (0.0)	10 (100.0)	8 (80.0)
PDTC	14	14 (100.0)	14 (100.0)	14 (100.0)	3 (21.4)	0 (0.0)
SCC	14	13 (92.9)	13 (92.9)	6 (42.9)	14 (100.0)	0 (0.0)
Thymus						
Epithelial cells	7	3 (42.9)	0 (0.0)	0 (0.0)	7 (100.0)	0 (0.0)
Lymphocytes	7	4 (57.1)	0 (0.0)	0 (0.0)	0 (0.0)	7 (100.0)
Thymoma	7	1 (14.3)	0 (0.0)	0 (0.0)	7 (100.0)	0 (0.0)
TC	1	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)

Abbreviations: ITTC, intrathyroid thymic carcinoma; p, polyclonal; m, monoclonal; PAX8, paired-box gene 8; TTF-1, thyroid transcription factor-1; PDTC, poorly differentiated thyroid carcinoma; SCC, squamous cell carcinoma; TC, thymic carcinoma.

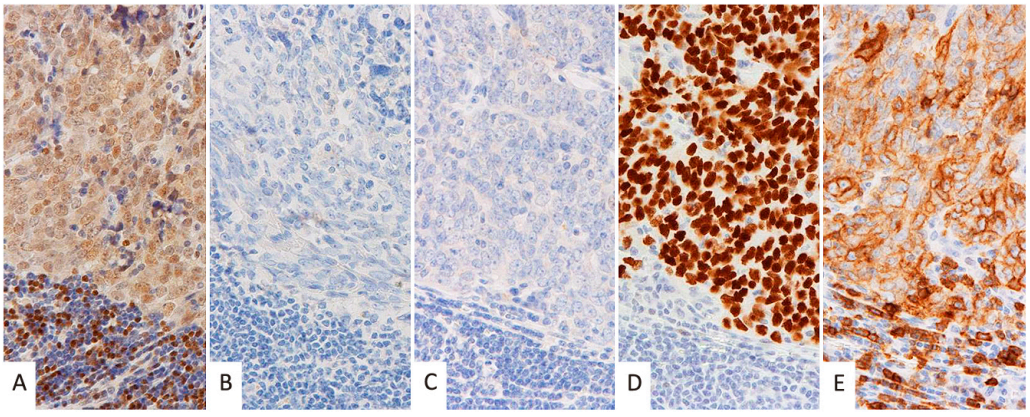

Fig. 1 Immunohistochemical staining of intrathyroid thymic carcinoma cells. (**A**) Polyclonal PAX8 (positive), (**B**) monoclonal PAX8 (negative), (**C**) thyroid transcription factor-1 (negative), (**D**) p63 (positive), and (**E**) CD5 (positive) (×20). Abbreviations: PAX8, paired-box gene 8

epithelial thymoma. In 2004, it was considered an independent clinicopathological entity in the World Health Organization classification, as carcinoma showing thymus-like differentiation of the thyroid [10] and renamed ITTC [1]. Although its microscopic appearance is similar to that of SCC or PDTC, ITTC is associated with a much more favorable prognosis than either SCC or PDTC [2, 11-13]. Therefore, ITTC should be distinguished from SCC or PDTC [2, 11, 14].

PAX8 is a member of the paired-box gene family comprising 9 genes (PAX1–9), all of which encode transcription factors [15]. PAX8 is expressed in the thyroid, kidney, ureter, ovary, uterus, and cervix [16]. Immunohistochemical staining has also shown PAX8 to be expressed in thymic tissue and carcinomas [8, 16-18]. Asirvatham *et al.* [16] reported that 69.2% of thymic carcinomas stained positive for PAX8. Weissferdt *et al.* [17] reported that 77% of thymic carcinomas were immunoreactive for PAX8, including 100% of type A and 93% of type B thymomas. Weak immunoreactivity was also detected in thymic epithelial cells [17]. However, these findings are difficult to accept given that PAX8 is not expressed in the thymic epithelium [19].

N-terminal regions of PAX family members have high sequence homology [20]. According to the report by Moretti *et al.* [20], the N-terminal regions PAX8 and PAX5 share 70% homology, which increases the possibility of cross-reactivity within this region. The epitope of monoclonal PAX8 antibody (clone: EPR13510) we used was the recombinant fragment within human PAX8 amino acid 150–300 [20]. Polyclonal PAX8 antibody can detect all 5 isoforms of PAX8 (31 kDa, 34 kDa, 41 kDa,

43 kDa, and 48 kDa), and may has cross-reactivities with other PAX family members [20, 21]. Therefore, there is a possibility that tumors without the PAX8 epitope could exhibit immunoreactivity for the polyclonal PAX8 antibody. Toriyama *et al.* [21] suggested that polyclonal PAX8 immunoreactivity in thymic carcinomas was likely to be the result of cross-reactivity with PAX5 or PAX6. They also reported that thymic tissue and carcinomas were not immunoreactive for monoclonal PAX8 [21].

To the best of our knowledge, ITTC immunoreactivity for PAX8 has not previously been reported. In the present study, we showed that 40.0% of ITTCs stained positive for polyclonal PAX8. However, none of the ITTCs were immunoreactive for monoclonal PAX8. The immunoreactivity of ITTCs for PAX8 was comparable to that of thymic carcinoma reported by Toriyama *et al.* [21]. We are the first to show that ITTCs are not immunoreactive for monoclonal PAX8. Monoclonal PAX8 is a more reliable marker than polyclonal PAX8 for determining follicular cell origin.

CD5 is a well-established positive marker for thymic carcinomas [22-24]. However, the positivity rate is not high, typically <40.0% [25]. Similarly, CD5 is a diagnostic marker for ITTC [23, 26]. It is useful for distinguishing ITTCs from PDTCs and SCCs [3, 27]. However, some ITTCs stain negative for CD5 antibody [5, 11]. According to a report by Ito *et al.* [11], the sensitivity of CD5 for ITTCs was 82.0%. In the present study, 2 ITTCs (20.0%) stained negative for CD5 antibody.

Based on the results of the present study, we recommend using both monoclonal PAX8 and CD5 antibodies for distinguishing ITTCs from PDTCs and SCCs. Monoclonal PAX8 positivity would suggest PDTC or SCC, while monoclonal PAX8 negativity and CD5 positivity would suggest ITTC.

We conclude that the monoclonal PAX8 antibody is a reliable marker for confirming follicular cell origin that is not expressed in ITTCs. Both monoclonal PAX8 and CD5 antibodies are useful for distinguishing ITTCs from PDTCs and SCCs. Monoclonal PAX8 negativity is additional evidence in support of ITTCs not being follicular cell-derived thyroid carcinomas, but having a thymic origin.

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Disclosure

The authors declare that they have no competing interests.

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