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
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 (0.9%) 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 46.2% 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).

**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...
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 [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.

References


