Diagnostic significance of PAX8 in thyroid squamous cell carcinoma

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Abstract. Most types of thyroid carcinomas express PAX8 transcription factor; however, whether thyroid squamous cell carcinoma (SCC) also expresses PAX8, currently remains unknown. We herein examined the immunoreactivity of PAX8 in SCC of thyroidal and extrathyroidal origin, and discussed the diagnostic significance of PAX8. We immunohistochemically examined specimens from 11 SCC, 22 papillary thyroid carcinoma (PTC), 8 anaplastic thyroid carcinoma (ATC), and 2 mucoepidermoid carcinoma (MEC) cases as well as 5 uterine cervical SCC, 5 esophageal SCC, and 5 pulmonary SCC cases. The rates of PAX8-positive SCC, PTC, ATC, and MEC were 90.9%, 90.9%, 75.0%, and 100%, respectively. Two PAX8-negative PTC cases were cribriform variants. No uterine cervical, esophageal, or pulmonary SCC specimen reacted with PAX8 antibody. Thyroid transcription factor-1 (TTF-1) was positive in 9.1% and 95.5% of SCC and PTC cases, respectively, but negative in all ATC and MEC cases. These results demonstrate that PAX8 staining is useful for distinguishing between primary thyroid SCC and invasion or metastasis from extrathyroidal SCC. We recommend using an immunohistochemical panel of antibodies to PAX8 and TTF-1 to confirm a diagnosis of primary thyroid carcinoma.

Key words: Thyroid, Immunohistochemistry, PAX8, Squamous cell carcinoma, Esophagus

PRIMARY SQUAMOUS CELL CARCINOMA (SCC) of the thyroid is microscopically composed entirely of carcinoma cells with squamous differentiation, and its incidence is very low [1]. SCC was previously shown to be a component of papillary thyroid carcinoma (PTC) or anaplastic thyroid carcinoma (ATC) [1]. These carcinomas are aggressive and frequently invade the surrounding organs, including the larynx, esophagus, and trachea. It is important to distinguish primary thyroid SCC from an invasion of SCC arising in the adjacent organs, because the prognosis of the former is worse than that of the latter [2]. However, difficulties are associated with preoperatively confirming a diagnosis of primary thyroid SCC in fine needle aspiration cytology or core needle biopsy samples. The presence of PTC or ATC may indicate primary thyroid SCC. Immunohistochemically, SCC cells generally express high-molecular-weight cytokeratin (HMW-CK), p63, and p53 [3]; however, an organ-specific marker has not yet been identified.

Paired box gene 8 (PAX8) is a transcription factor that is essential for embryonic development of the kidney, Müllerian organs, and thyroid [4]. The expression of PAX8 was recently detected immunohistochemically in carcinomas arising in these organs [4]. In the thyroid, PAX8 was shown to be immunopositive for PTC, follicular thyroid carcinoma (FTC), poorly differentiated thyroid carcinoma (PDTC), medullary thyroid carcinoma (MTC), and even ATC [5, 6]. To the best of our knowledge, it has yet to be determined whether thyroid SCC expresses PAX8. Therefore, we herein examined the immunoreactivity of PAX8 in thyroidal and extrathyroidal SCC, and discussed the diagnostic significance of PAX8.

Materials and Methods

We searched the pathology report database of 8,365 cases of thyroid carcinoma that were operated on in
Results

Primary thyroid carcinomas

38 (88.4%) out of 43 thyroid carcinoma cases were positive for PAX8, and in 35 (81.4%) of them more than 50% of carcinoma cells showed the immunopositivity for the antibody. Ten (90.9%) out of 11 SCC cases showed nuclear positivity for PAX8 (Fig. 1A) (Table 2). In some cases, the foci, in which the nuclei and cytoplasm were both equally stained, were observed. We regarded this phenomenon as false positive. Twenty (90.9%) out of 22 PTC cases were PAX8-negative PTC cases were the cribriform variant. Squamous metaplasia was positive for the antibody (Fig. 2A). The PAX8-positive rate of morules in cribriform variant PTC was 14.3%.

One (9.1%) out of 11 SCC cases and 21 (95.5%) out of 22 PTC cases were positive for thyroid transcription factor-1 (TTF-1) (Fig. 1B). The SCC case and 2 ATC cases without PAX8 expression were both included among TTF-1-negative cases. The TTF-1-positive rate in the foci of squamous metaplasia was 50.0% (Fig. 2B).

Kuma Hospital between 2008 and 2014, and 11 cases that had the SCC component in at least a part of the tumor were extracted. The diagnosis of SCC required apparent carcinoma cells with squamous differentiation, which was identified by the presence of keratinization, an intercellular bridge, or stratification. We reviewed these cases, and confirmed that 3 out of 11 of them were composed of only the SCC component. The remaining eight cases were associated with PTC or ATC. We also extracted 22 PTC, 8 ATC, and 2 mucoepidermoid carcinoma (MEC) cases in the same manner. Ten and seven out of the 22 PTC cases were PTC with squamous metaplasia and the cribriform variant with morula, respectively. The remaining cases were conventional PTC. We also examined 5 uterine cervical SCC, 5 esophageal SCC, and 5 pulmonary SCC cases that were collected from the pathology files of Oita University Hospital.

We performed immunostaining using 3-µm-thick, formalin-fixed, paraffin-embedded tissue. The primary antibodies used in the immunostaining and antigen retrieval methods were shown in Table 1. Staining was carried out using the Leica Bondmax system (Leica Microsystems IL) and Bond refine kit (Leica Microsystems, IL) according to the manufacturer’s recommendation. The positive decision criteria of immunostaining were defined as cases with more than 10% carcinoma cells expressing moderate or strong positive staining [7].

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Vendor</th>
<th>Location</th>
<th>Antigen retrieval</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX8</td>
<td>polyclonal</td>
<td>Protein Tech</td>
<td>Chicago, IL, USA</td>
<td>Heat (pH6)</td>
<td>1:200</td>
</tr>
<tr>
<td>TTF-1</td>
<td>8G7G3/1</td>
<td>Dako</td>
<td>Carpinteria, CA, USA</td>
<td>Heat (pH6)</td>
<td>1:100</td>
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<tr>
<td>p63</td>
<td>4A4</td>
<td>Nichirei Biosciences</td>
<td>Tokyo, Japan</td>
<td>Heat (pH9)</td>
<td>(-)</td>
</tr>
<tr>
<td>HMW-CK</td>
<td>34βE12</td>
<td>Dako</td>
<td>Carpinteria, CA, USA</td>
<td>Protein K</td>
<td>1:3</td>
</tr>
</tbody>
</table>

Table 1 Primary antibodies used in immunostaining and antigen retrieval methods

| Table 2 Immunohistochemical results of thyroid carcinomas |
|-----------------|-----------------|-----------------|-----------------|
| Antibody     | PAX8 (90.9%) | TTF-1 (95.5%) | p63 (50.0%) | HMW-CK (62.5%) |
| SCC (11)  | 10 (100%) | 1 (100%) | 1 (100%) | 1 (100%) |
| PTC (22) | 20 (90.9%) | 21 (95.5%) | 1 (4.5%) | 0 (0%) |
| SM (10)  | 8 (80.0%) | 5 (50.0%) | 8 (80.0%) | 9 (90.0%) |
| Morules (7) | 1 (14.3%) | 0 (0%) | 0 (0%) | 0 (0%) |
| ATC (8)  | 6 (75.0%) | 2 (25.0%) | 5 (62.5%) | 5 (62.5%) |
| MEC (2)  | 2 (100%) | 0 (0%) | 2 (100%) | 2 (100%) |

SCC, squamous cell carcinoma; PTC, papillary thyroid carcinoma; SM, squamous metaplasia; ATC, anaplastic thyroid carcinoma; MEC, mucoepidermoid carcinoma; TTF-1, thyroid transcription factor-1; HMW-CK, high-molecular-weight cytokeratin.

TTF-1, thyroid transcription factor-1; HMW-CK, high-molecular-weight cytokeratin.
Fig. 1 Squamous cell carcinoma. Most carcinoma cells were moderately positive for PAX8 (A). TTF-1 was negative (B). Carcinoma cells located at the periphery of the nests were positive for p63 (C). High-molecular-weight cytokeratin reacted with keratinized carcinoma cells (D).

Fig. 2 Papillary carcinoma (left) associated with squamous metaplasia (right). Papillary carcinoma cells were positive for PAX8 (A) and TTF-1 (B), and negative for p63 (C) and high-molecular-weight cytokeratin (D). Squamous metaplastic cells were positive for PAX8 (A), p63 (C) and high-molecular-weight cytokeratin (D), and negative for TTF-1 (B).
mary thyroid SCC reacts with PAX8 has not yet been determined. Regarding the expression of PAX8 in PTC and ATC, our results were similar to previous findings. We demonstrated that the squamous metaplasia component associated with PTC, MEC, and primary SCC of the thyroid, which showed squamous differentiation, were also positive for PAX8.

Primary thyroid SCC expresses PAX8. Our results showed that SCC of the uterine cervix, esophagus, and lung did not react with PAX8. Ozcan et al. reported that SCC of the uterine cervix, lung, larynx, and skin did not express PAX8 [4]. SCC of the esophagus, lung, larynx, and skin do not express PAX8 because these organs do not naturally have PAX8 [4]. SCC of the uterine cervix and thyroid, both of which are organs expressing PAX8, showed different results; the former were negative while the latter positive. These results demonstrated that PAX8 staining was useful for distinguishing between primary thyroid SCC and invasion or metastasis from extrathyroidal SCC, especially in preoperative fine needle aspiration cytology or core needle biopsy, which cannot examine throughout the carcinoma.

In our study, differences were noted in the expression of PAX8 between cribriform variant PTC and conventional PTC. The latter invariably showed diffuse and strong positivity for the antibody. Staining of the former was weak and inhomogeneous. Only two PAX8-negative PTC cases were the cribriform variant. Squamous metaplasia was positive for PAX8, while the PAX8-positive rate of morules, which are characteristic of cribriform variant PTC and superficially similar to squamous metaplasia, was 14.3%. The results of the present study support morules not being an early form of squamous metaplasia [8].

As with PAX8, TTF-1 is also a transcription factor. TTF-1 was previously shown to be a transcriptional regulator of thyroid-specific genes, and is also involved in the activation of lung-specific differentiation-inducing genes [9]. In normal tissue, thyroid follicular cells, renal tubular epithelial cells, and epithelial cells of the fallopian tubes, endometrial glands, epididymis, vas deferens, and seminal vesicles have been shown to express PAX8 [4]. Furthermore, tumors arising in the Müllerian organs, kidney, and thyroid were found to maintain the expression of PAX8 [4].

In surgical pathology, the study of PAX8 has been limited. Nonaka et al. demonstrated that PTC, FTC, and PDTC invariably expressed PAX8 [5]. The expression of PAX8 was also detected in metastatic lesions [4]. Previous studies reported that PAX8 was mostly positive for ATC [5, 6]. Bishop et al. reported that PAX8 staining was a reliable means of discriminating a thyroid origin for undifferentiated tumors of the head and neck [6]. They demonstrated that all squamous ATC cases expressed PAX8. However, whether primary thyroid SCC reacts with PAX8 has not yet been determined. Regarding the expression of PAX8 in PTC and ATC, our results were similar to previous findings. We demonstrated that the squamous metaplasia component associated with PTC, MEC, and primary SCC of the thyroid, which showed squamous differentiation, were also positive for PAX8.

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### Table 3 Immunohistochemical results of extrathyroidal squamous cell carcinomas

<table>
<thead>
<tr>
<th></th>
<th>PAX8</th>
<th>TTF-1</th>
<th>p63</th>
<th>HMW-CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine cervix</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Lung</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
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TTF-1, thyroid transcription factor-1; HMW-CK, high-molecular-weight cytokeratin.

### Extrathyroidal carcinomas

All uterine cervical, pulmonary, and esophageal SCC cases were negative for PAX8 and TTF-1. P63 and HMW-CK were strongly positive (Table 3).

### Discussion

PAX8 is known to control the development of the central nervous system, eye, kidney, thyroid gland, and organs derived from the Müllerian duct [4]. In normal tissue, thyroid follicular cells, renal tubular epithelial cells, and epithelial cells of the fallopian tubes, endometrial glands, epididymis, vas deferens, and seminal vesicles have been shown to express PAX8 [4]. Furthermore, tumors arising in the Müllerian organs, kidney, and thyroid were found to maintain the expression of PAX8 [4].

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by thyroid organogenesis. In view of the chronological emergence of transcription factors, PAX8 was identified as the earliest transcription factor, followed by TTF-1 [5]. Therefore, we considered the expression of PAX8 to be retained, even in PDTC, ATC, and SCC.

We herein clearly demonstrated that PAX8 was immunohistochemically positive for primary thyroid SCC and negative for extrathyroidal SCC. Therefore, PAX8 staining is considered useful for distinguishing between primary thyroid SCC and invasion or metastasis from extrathyroidal SCC. We recommend an immunohistochemical panel using PAX8 and TTF-1 to confirm a diagnosis of primary thyroid carcinoma. The expression of PAX8 may exclude TTF-1-positive pulmonary adenocarcinoma, whereas that of TTF-1 may exclude PAX8-positive carcinomas of the kidney and female genital tracts.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

References