Original Article

Immunohistochemical differentiation between type B3 thymomas and thymic squamous cell carcinomas

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Abstract: Type B3 thymomas and thymic squamous cell carcinomas have some overlapping histological features, so it is difficult to make the differential diagnosis between these two entities, especially when the biopsy specimen is small. Only a few markers such as CD5 and CD 117 were applied to the differential diagnosis, the purpose of this study is to identify other diagnostic markers to help making the differential diagnosis more accurate. GLUT-1, MUC-1, CD117, CD5, CE, P63, CK19, CK5/6, CD1a and TdT were evaluated using 16 cases of type B3 thymoma and 20 cases of thymic squamous cell carcinoma. Staining scores were obtained by calculating the percentage of positive cells. The sensitivity of GLUT-1 or MUC-1 for thymic squamous cell carcinomas was highest (100%), followed by CK5/6 (95%), CD117 (90%), P63 (85%), CD5 (80%) and CEA (75%). The specificities of CD5, CD117 and CEA for thymic squamous cell carcinomas all were 100%, next was MUC-1 (69.3%), followed by GLUT-1 (50%), P63 (25%), CK5/6 (12.5%). The sensitivities of CK19, TdT, and CD1a for type B3 thymomas were 100%, 93.8% and 87.5%, respectively. The specificity of CD1a for type B3 thymomas was highest (100%), followed by TdT (95%), CK19 (90%). The reactivity of GLUT-1, MUC-1, CD117, CD5, CE, CD1a and TdT in thymic squamous cell carcinomas and type B3 thymomas had significant difference. Usually a panel of markers is needed, if we combine GLUT-1 or MUC-1 which sensitivity for thymic squamous cell carcinomas is highest with CD5, CD117, CE, CD1a or TdT which have high specificity, we can make the differential diagnosis effectively.

Keywords: Type B3 thymoma, thymic squamous cell carcinoma, immunohistochemistry, differentiation

Introduction

In 2004, the World Health Organization (WHO) classified epithelial thymic tumors into type A, AB, B1, B2, B3 thymoma and thymic carcinoma [1]. Type B3 thymoma is predominantly epithelial type, with a few immature T lymphocytes. The tumor cells usually palisade around the perivascular space, with mild to moderate cellular atypia. Foci of squamous metaplasia could be found in some cases. Thymic carcinomas usually have obvious cellular atypia, with a small number of mature lymphocytes and plasma cells. Thymic carcinomas include several histological types which are similar to the same histological types of extrathymic carcinomas without organotypical features of thymic differentiation. Squamous cell carcinoma is the most frequent type [1]. Either type B3 thymoma or thymic carcinoma may have obvious atypia. Sometimes it is difficult to make the differential diagnosis between them histologically, especially between type B3 thymoma and squamous cell carcinoma, particularly when the biopsy specimen is small [2]. Some markers such as CD5 and CD 117 were used to the differential diagnosis, however, the positive rate of CD5 and CD117 in thymic carcinoma had been reported to be 50%-70%, 50%-90%, respectively [3-7]. Moreover, not all neoplastic cells in thymic carcinomas were stained positive for these markers. Meanwhile, the tumor cells in a few number of type B3 thymomas could be positive for CD5 or CD 117 [8, 9]. So it is necessary to identify other diagnostic markers to help the differential diagnosis.

GLUT-1, CEA and MUC-1 were reported to be useful in the differential diagnosis between type B3 thymomas and thymic carcinomas recently [2, 10]. However only a few studies...
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were related to these markers and the cases involved in these studies were very limited, so the value of these markers needs to be further analyzed. As we know, CD1a and TdT are always stained positive for the immature T cells in type B3 thymomas, CK5/6 and P63 are usually positive for squamous cell carcinomas, if these markers could help to make the differential diagnosis more reliable? In this study, we evaluated a panel of antibodies and try to choose the markers which can help the differentiation of thymic squamous cell carcinomas (TSCCs) and type B3 thymomas.

Materials and methods

Subjects

Two hundred and forty-nine surgically treated cases of thymic epithelial tumors were collected from the Department of Pathology, West China Hospital of Sichuan University since 1999 to 2009. According to the WHO 2004 classification schema, there were 18 cases of type A, 97 of type AB, 22 of type B1, 63 of type B2, 16 of type B3 and 33 of thymic carcinoma. The thymic carcinomas consisted of 8 cases of keratinizing squamous carcinomas, 13 cases of nonkeratinizing carcinomas, 6 cases of neuroendocrine carcinomas, 4 cases of lymphoepithelioma-like carcinomas, 1 sarcomatoid carcinoma and 1 adenocarcinoma. 16 cases of type B3 thymoma and 20 cases of thymic squamous cell carcinoma were included in this study.

The specimens were fixed with 10% neutral buffered formalin and embedded in paraffin. 4-6 μm sections were stained using hematoxylin and eosin (H&E). All the cases were reviewed and reclassified according to the 2004 WHO classification system by two pathologists. Some complicated cases were reviewed with the famous German pathologist Dr. Müller-Hermelink using a multiheaded microscope. The discrepancies were resolved by joint discussion. The diagnosis of TSCC was made after excluding metastases from other sites. Representative features of type B3 thymoma and TSCC were shown in Figure 1.

Immunohistochemistry

All the primary antibodies used in this study were listed in Table 1. The immunostaining was performed on 4 μm paraffin sections for each case. The slides were deparaffinized in xylene, then rehydrated through graded alcohol series. The endogenous peroxidase was blocked using 3% hydrogen peroxide. Slides were immunostained with the primary antibodies shown in Table 1. The sections were washed with PBS and stained using DAKO EnVision + (Dako, Carpinteria, CA, USA) system. Finally the slides were counterstained with hematoxylin.

Only membrane based reactivity was regarded as positive for CD1a, CD5 and CD117, cytoplasm for CEA, CK5/6 and CK19, both membrane and cytoplasm for MUC-1, GLUT-1, nuclear based reactivity was regarded as positive for P63 and TdT.

For GLUT-1, MUC-1, CD117, CD5, CEA, P63, CK19 and CK5/6, the immunohistochemical stains were recorded as positivity when >10% of tumor cells showed positive. For CD1a and TdT, the immunohistochemical stains were recorded as positivity when >10% of lymphocytes showed positive. Staining scores were made by calculating the percentage of positive cells per slide: 0-10% (negative), 10%-25% (weak), 25%-50% (moderate) and >50% (strong).

Statistical analysis

SPSS17.0 system was used for statistical analysis. The results of immunohistochemical staining were evaluated by the χ²-test or Fisher’s exact test. A significant level of 0.05 was used, P-value <0.05 was considered as significant difference.

Results

Clinical pathological features

The ages of 16 cases of type B3 thymoma ranged from 26 to 76 years old (median =43.5), male to female ratio was 1.67:1. All the tumors were located in the anterior mediastinum in which 7 cases were in the anterosuperior mediastinum. The largest tumor dimension ranged from 3.5 cm to 13 cm (mean =8.3 cm). The Perivascular spaces with palisading of the tumor cells were found in 12 cases. Foci of squamous metaplasia presented in 8 cases in which 2 cases had keratinizing changes. This group of B3 thymomas included 1 of Masaoka
Differentiation of type B3 thymoma and TSCC

The ages of 20 cases of TSCC ranged from 20 to 70 years old (median =52.5), male to female ratio was 1:1. 19 cases were located in the anterior mediastinum in which 14 cases were in the anterosuperior mediastinum, 1 case was in middle mediastinum. The largest tumor dimension ranged from 4.3 cm to 12 cm (mean =8.4 cm). None presented organotypical features related to thymic differentiation. There were 8 cases of keratinizing squamous cell carcinoma and 12 cases without keratinization.

I, 6 of Masaoka II, 6 of Masaoka III and 3 of Masaoka IV.

This group of thymic carcinomas included 16 of Masaoka III and 4 of Masaoka IV, none was in Masaoka I or Masaoka II.

Immunohistochemical studies

CD5: 16 cases of TSCC reacted with CD5 (Figure 2A), none of the type B3 thymomas was positive for CD5. The sensitivity of CD5 for TSCCs was 80% while the specificity was 100%. The reactivity of CD5 in TSCCs and type B3 thymomas had significant difference (P=0.000).

CD117: 18 cases of TSCC were stained positive for CD117 (Figure 2B), none of the type B3 thymomas reacted with it. The sensitivity of CD117 for TSCCs was 90% while the specificity was 100%. The reactivity of CD117 in TSCCs and type B3 thymomas had significant difference (P=0.000).

GLUT-1: 20 cases of TSCC were all strongly positive for GLUT-1 (Figure 2C) while only 8 cases of type B3 thymoma reacted with it. Only one type B3 thymoma presented as strong positivity, the others were recorded as weakly positive. The positive signals tended to be more intense in the central area of the tumor nests than in the peripheral area (Figure 2D). The sensitivity of GLUT-1 for TSCCs was 100% while the specificity was 50%. The reactivity of GLUT-1 in

Table 1. Antibody information

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
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<th>Ag retrieval</th>
<th>Dilution</th>
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<td>EDTA</td>
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<td>Dako</td>
<td>–</td>
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<tr>
<td>CEA</td>
<td>CEA-31</td>
<td>ZYMED</td>
<td>PC</td>
<td>1:100</td>
</tr>
<tr>
<td>GLUT-1</td>
<td>Polyclone</td>
<td>Maxim</td>
<td>PC</td>
<td>1:100</td>
</tr>
<tr>
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<td>MRQ-17</td>
<td>ZYMED</td>
<td>PC</td>
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<td>D5/16B4</td>
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<td>EDTA</td>
<td>Prediluted</td>
</tr>
<tr>
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<td>ZYMED</td>
<td>EDTA</td>
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</tr>
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<td>RCK108</td>
<td>Dako</td>
<td>PC</td>
<td>1:100</td>
</tr>
<tr>
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<td>010</td>
<td>Dako</td>
<td>EDTA</td>
<td>1:100</td>
</tr>
<tr>
<td>TdT</td>
<td>SEN28</td>
<td>Maxim</td>
<td>EDTA</td>
<td>1:100</td>
</tr>
</tbody>
</table>

Ag: Antigen; PC: Pressure cooker.

Figure 1. Representative features of type B3 thymomas and thymic squamous cell carcinomas. A. Type B3 thymoma with a few immature T cells around the tumor cells, HE×400. B. Type B3 thymoma with squamous metaplasia and infiltration, HE×200. C. Type B3 thymoma with obvious atypia and active mitosis, HE×400. D. TSCC with invasive growth, HE×100. E. TSCC with some lymphocytes, HE×400. F. TSCC with keratinization, HE×400.
TSCCs and type B3 thymomas had significant difference ($P=0.000$).

**MUC-1**: 20 cases of TSCC were all stained positive for MUC-1 (Figure 2E) and 15 cases were strongly positive. Only 7 cases of type B3 thymoma reacted with MUC-1 in which 5 cases showed weakly positive and 2 cases were moderate positive (Figure 2F). The sensitivity of MUC-1 for TSCCs was 100% while the specificity was 56.3%. The reactivity of MUC-1 in TSCCs and type B3 thymomas had significant difference ($P=0.000$).

**CEA**: 15 cases of TSCC were positive for CEA (Figure 3A) while all of type B3 thymomas were
Negative for it. The sensitivity of CEA for TSCCs was 75% while the specificity was 100%. The reactivity of CEA in TSCCs and type B3 thymomas had significant difference ($P=0.000$).

**P63:** 17 cases of TSCC were positive for P63 (Figure 3B) and 12 cases of type B3 thymomas reacted with it. The sensitivity of P63 for TSCCs was 85% while the specificity was only 25%. We couldn’t conclude the reactivity of P63 in TSCCs and type B3 thymomas had significant difference ($P=0.369$).

**CK5/6:** 19 cases of TSCC were positive for CK5/6 while 14 cases of type B3 thymomas reacted with it (Figure 3C). The sensitivity of...
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CK5/6 for TSCCs was 95% while the specificity was only 12.5%. We couldn’t conclude the reactivity of CK5/6 in TSCCs and type B3 thymomas had significant difference ($P=0.415$).

CK19: 16 cases of TSCC were positive for CK19 while 18 cases of type B3 thymomas reacted with it (Figure 3D). The sensitivity of CK19 for TSCCs was 100% while the specificity was only 10%. We couldn’t conclude the reactivity of CK19 in TSCCs and type B3 thymomas had significant difference ($P=0.302$).

CD1a: The lymphocytes in 16 cases of type B3 thymomas were positive for CD1a (Figure 3E) while the lymphocytes in all TSCCs were negative for it. The sensitivity of CD1a for type B3 thymomas was 87.5% while the specificity was 100%. The reactivity of CD1a in TSCCs and type B3 thymomas had significant difference ($P=0.000$).

TdT: The lymphocytes in 15 cases of type B3 thymomas were positive for TdT while the lymphocytes in only one TSCC were positive for it (Figure 3F). The sensitivity of TdT for type B3 thymomas was 93.8% while the specificity was 95%. The reactivity of TdT in TSCCs and type B3 thymomas had significant difference ($P=0.000$).

The reactivity of the above antibodies was summarized in Table 2.

Discussion

Thymic squamous cell carcinoma (TSCC) is the most common histological type of thymic carcinoma. Sometimes it is hardly to be distinguished from type B3 thymoma only based on HE stained slides because of the overlapping morphological features. In this study, we selected 20 surgically removed TSCCs and 16 type B3 thymomas to evaluate the immunohistochemical differential diagnosis between them.

CD5 and CD117 are usually applied to the differential diagnosis between these two entities. In our study, the sensitivities of CD5 and CD117 for TSCC were 80% and 100%, respectively. The specificities of CD5 and CD117 for TSCCs both were 100%. We thought these two markers were very useful. But there were four cases of TSCC stained negative for CD5, two cases of TSCC were negative for CD117. For one case, the tumor cells didn’t react with both markers, so we thought some other markers were needed to help making a correct diagnosis.

Glucose transporter 1 (GLUT-1) is one of 14 members of the mammalian facilitative glucose transporter family of passive carriers that function as an energy independent system for transport of glucose down a concentration gradient [11, 12]. It is thought to be a possible intrinsic marker of hypoxia. GLUT-1 is expressed in a variety of malignant tumors including renal cell carcinoma, breast carcinoma, ovarian carcinoma, lung cancer and malignant pleural mesothelioma [13-15]. The study about the expression of GLUT-1 in thymic tumors is very limited. Kojika et al. reported the sensitivity and specificity of GLUT-1 for thymic carcinomas were 70% and 100%, respectively [2]. However in a research of Hayashi’s, GLUT-1 was found to be not very useful for the differential diagnosis because of the low specificity [16]. In our study, all the TSCCs were strongly positive for GLUT-1, the sensitivity was 100%. Though 8 cases of type B3 thymoma also reacted with it, the signals in 7 cases were weak. Moreover, the positive signals of GLUT-1 for type B3 thymomas were more intense in the central area of the tumor nests than in the peripheral area. Although the specificity was not so high, the staining pattern in type B3 thymomas was different from TSCCs, so we thought GLUT-1 was helpful to the differential diagnosis.

The transmembrane mucin MUC-1 is a heavily O-glycosylated protein expressed on most

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Table 2. Summary of the antibodies reactivity

<table>
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<tr>
<th>Antibody</th>
<th>CD5</th>
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<th>CK5/6</th>
<th>P63</th>
<th>GLUT-1</th>
<th>MUC-1</th>
<th>CK19</th>
<th>CD1a</th>
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<tbody>
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<td>TSCC</td>
<td>+</td>
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<td>-</td>
<td>+</td>
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<td>+</td>
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<td>0</td>
</tr>
<tr>
<td>B3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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TSCC: thymic squamous cell carcinomas, B3: type B3 thymomas.
secretory epithelium. MUC-1 consists of a heavily O-glycosylated extracellular domain, a transmembrane domain and a cytoplasmic tail of 72 amino acids [17, 18]. It was reported that MUC-1 oncoprotein aberrantly expressed at high levels in most human neoplasms, and MUC-1 played important roles in development and progression of malignant tumors [19-21]. The precise function of MUC-1 overexpression in tumorigenesis is still unknown, although various domains of MUC-1 had been implicated in cell adhesion, cell signaling, and immunoregulation. The overexpression of MUC-1 had been also reported to be associated with bad prognosis in many malignant tumors [20, 22]. There are only a few studies related to the expression of MUC-1 in thymic epithelial tumors. Kaira et al. reported MUC-1 was stained positive in 94% (16/17) thymic carcinomas, all type B3 thymomas in his study were negative for MUC-1 [10]. In another research, the sensitivity and specificity of MUC-1 for thymic squamous cell carcinomas were 33% and 100%, respectively [2]. In our study, all the TSCCs were positive for MUC-1, the sensitivity was 100%. 7 cases of type B3 thymoma weakly reacted with it and the specificity was 56.3%. The sensitivity in our study was higher than which was reported in the literatures, but the specificity was not so high. We found 75% (15/20) TSCCs was strong positive for MUC-1 while all the thymomas were weakly positive, so we thought MUC-1 was also useful to the differential diagnosis.

CEA was found as a marker for colon adenocarcinoma. The positive rate of CEA was higher in gastrointestinal adenocarcinoma, the adenocarcinoma of lung; breast and ovary also could express it in varied degree [23-25]. Kojika et al. tried to use CEA as the marker for thymic carcinoma. There were 6 cases of keratinizing carcinoma and 6 cases non-keratinizing carcinoma in his study, only 3 cases of thymic carcinoma stained positive for it, the sensitivity was only 25% while the specificity was 100% [2]. In our study, the sensitivity of CEA was 75%, none of type B3 thymoma reacted with it. If CEA was combined with CD5 and CD117, the sensitivity for TSCCs reached 100%.

CK5/6 or P63 was often used as the marker for squamous cell carcinomas. But only a few studies were related to the expression of CK5/6 or P63 in TSCC. Kojika et al. revealed CK5/6 was useless in the differential diagnosis of TSCC and thymoma [2]. However, Khoury’s research showed that CK5/6 was one of the best markers for the differential diagnosis. The sensitivity and specificity of CK5/6 for TSCCs were 75% and 88.2%, respectively [8]. In our study, the sensitivity of CK5/6 for TSCCs was 95%. Though the sensitivity was even higher than which was reported, the specificity was only 12.5%, we didn’t think CK5/6 was very useful. Khoury showed 100% (17/17) TSCCs reacted with P63, the sensitivity was 100%, but the specificity was only 8.3% [8]. In our study, the sensitivity and specificity of P63 for TSCCs were 85% and 25%, respectively. The value of P63 for the differential diagnosis was questionable.

Kuo et al. found all thymic epithelial cells were stained positive for CK19 regardless of different epithelial compartments [26]. However, four thymomas studied by Savino and Dardenne were all negative for CK19 [27]. In a study of Fukai et al., it was revealed that different types of thymoma could not be distinguished by the CK expression patterns [28]. Kojika et al. concluded the expression of CK19 hadn’t significant difference in type B3 thymomas and thymic carcinomas [2]. In our study, 100% (16/16) type B3 thymomas were stained positive for CK19 and 90% (18/20) TSCCs reacted with it. We thought CK19 was not useful to the differential diagnosis.

The immature T lymphocytes in type B3 thymoma were characterized by CD1a positive and TdT positive. CD1a and TdT were previously found to can help to differentiate thymoma from thymic carcinoma. This finding was also consistent with our study. There were 87.5% of type B3 thymomas stained positive for CD1a, 93.8% for TdT. The lymphocytes in all the TSCCs were negative for CD1a. For one TSCC, the lymphocytes were positive for TdT, the specificity was 95% (19/20). We thought CD1a and TdT were both useful markers due to the high sensitivity and specificity.

In conclusion, the sensitivity of GLUT-1 or MUC-1 for TSCC was highest (100%), followed by CK5/6 (95%), CD117 (90%), P63 (85%), CD5 (80%) and CEA (75%). The specificities of CD5, CD117 and CEA for TSCCs all were 100%, next was MUC-1 (56.3%), followed by GLUT-1 (50%), P63 (25%), CK5/6 (12.5%). The sensitivities of CK19, TdT, and CD1a for type B3 thymomas
were 100%, 93.8% and 87.5%, respectively. The specificity of CD1a for type B3 thymomas was highest (100%), followed by TdT (95%), CK19 (10%). The reactivity of GLUT-1, MUC-1, CD117, CD5, CEA, CD1a and TdT in TSCCs and type B3 thymomas had significant difference. A panel of markers is needed for the differential diagnosis. If we combine GLUT-1 or MUC-1 which sensitivity for TSCC was highest with CD5 or CD117, CEA, CD1a or TdT which have high specificity, we can make the differential diagnosis effectively.

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Disclosure of conflict of interest

None.

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References

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