An Immunohistologic Study of the Epithelial Components of 81 Cases of Thymoma

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Eighty-one cases of thymoma were studied immunohistologically with the use of three mouse monoclonal antibodies: one was specific for subcapsular-cortical, one for intra-cortical, and one for medullary epithelial cells. Twenty-eight (60.9%) of 46 polygonal cell thymomas were of the cortical type and 1 (2.2%) was of the medullary type. Ten (55.6%) of 18 spindle cell thymomas and 7 (41.2%) of 17 mixed cell thymomas were of the medullary type, and 1 (5.6%) of 18 spindle cell thymomas was of the cortical type. Fourteen (17.3%) of 81 thymomas were composed of epithelial cells that were triple positive immunologically; although these are unusual, they also may be present in the normal thymus. Based on these findings, triple-positive epithelium in the normal thymus consists of common stem cells that can differentiate into subcapsular-cortical, intra-cortical, and medullary epithelium; these cells may be the target cells for tumorigenesis. Epithelium in polygonal cell thymoma tends to differentiate into cortical epithelium, whereas epithelium in spindle and mixed cell thymomas differentiates into medullary epithelium. Cancer 1992; 69:2463-2468.

In the normal human thymus, three distinct epithelial cells—subcapsular-cortical, intra-cortical, and medul-
medium (Miles Incorporated, Elkhart, IN) and stored at 
−75°C for immunoperoxidase staining. Portions of the 
specimens fixed in 10% formalin were embedded in 
paraffin for routine histologic studies. Based on the 
shape of the neoplastic epithelial cell and the criteria of 
Rosai and Levine, the 81 thymomas were classified as 
follows: polygonal, 46; spindle, 18; and mixed cell, 17 
(Fig. 1).

Reagents

Three mouse monoclonal antibodies were used: anti-
Leu-7 (Becton Dickinson, San Jose, CA), UH-1, and 
PE-35.

Staining Procedure

Frozen serial sections (4 μm) were fixed in acetone at 
4°C for 10 minutes, air-dried, washed in phosphate-
buffered saline (pH 7.2), and then stained according to 
immunoperoxidase staining techniques. Sections were 
stained according to the streptavidin–biotin–peroxi-

Results

Reactivity of Antithymic Epithelial Antibodies With 
the Normal Thymus

UH-1 reacted with cortical epithelium by forming a net-
work (Fig. 2, top left). Subcapsular-cortical epithelium, 
which was negative for UH-1 (Fig. 2, top left), was posi-
tive for anti-Leu-7. A small group of Leu-7-positive 
epithelium also was observed in the outer cortex in a

Figure 1. Three histologic types of thymoma. (Top left) 
Polygonal cell type (H & E, original magnification ×100). (Top 
right) Spindle cell type (H & E, original magnification ×100). 
(Bottom) Mixed cell type (H&E, original magnification ×100).
serial section (Fig. 2, top right). PE-35 reacted with medullary epithelium (Fig. 2, bottom).

Reactivity of Antithymic Epithelial Antibodies With Thymomas of Three Different Histologic Types

The staining patterns of individual thymomas fell into two major categories: thymomas with epithelial networks that only stained for one marker and thymomas with epithelial networks that were positive for two or three markers. Because we used serial sections in the current study, three markers were found to be expressed in various combinations on the same neoplastic epithelium (Fig. 3). We recognized eight immunologic phenotypes of neoplastic epithelium: U (UH-1+, PE-35−, Leu-7−); P (UH-1−, PE-35+, Leu-7−); L (UH-1−, PE-35−, Leu-7+); UP (UH-1+, PE-35+, Leu-7−); UL (UH-1+, PE-35−, Leu-7+); PL (UH-1−, PE-35+, Leu-7+); UPL (UH-1+, PE-35+, Leu-7+); and N (UH-1−, PE-35−, Leu-7−). The relationship between the histologic types and the immunologic phenotypes is summarized in Table 1. Epithelium of phenotypes U, P, and L is common in the normal thymus, whereas epithelium of phenotypes UP, UL, PL, UPL, and N is not. Epithelium of phenotype UL appears to be localized in the cortex. Therefore, epithelium of phenotypes U, L, and UL can be classified as the immunologically cortical type. Twenty-eight (60.9%) of 46 polygonal cell thymomas were of the cortical type and consisted of the cortical type of epithelium, whereas 10 (55.6%) of 18 spindle and 7 (41.2%) of 17 mixed cell thymomas were of the medullary type and consisted of the medullary type of epithelium, i.e., epithelium of phenotype P. Of 46 polygonal cell thymomas, only 1 (2.2%) was of the medullary type. Of 18 spindle cell thymomas, only 1 (5.6%) was of the cortical type. There was no cortical type in 17 mixed cell thymomas.

Histologic Types and Immunologic Phenotypes of Thymoma With MG

MG was observed in 29 (35.8%) of 81 thymomas. The histologic types and immunologic phenotypes of these 29 thymomas are summarized in Table 2. They consisted of 24 polygonal and 5 mixed cell thymomas. Six-
Figure 3. Localization of (top left) UH-1, (top right) PE-35, and (second row, left) Leu-7 in polygonal cell thymoma from the same patient. UH-1-positive epithelium forms a major network and both PE-35-positive cells and Leu-7-positive cells create a complete network. Localization of (second row, right) PE-35 and (third row, left) Leu-7 in a spindle cell thymoma from the same patient showing a complete positive network. Localization of (third row, right) PE-35 and (bottom) Leu-7 in a mixed cell thymoma from the same patient showing a complete positive network (streptavidin–biotin–peroxidase complex method, original magnification ×50).
Immunologic Phenotypes of Thymomas/Fukai et al.

Discussion

In the normal human thymus, three distinct epithelial cells—subcapsular-cortical, intra-cortical, and medullary—can be distinguished based on their localization and reactivity to various markers. Thus, thymomas may be classified into at least three types depending on which epithelial cells they are derived from (subcapsular-cortical, intra-cortical, or medullary). However, an analysis of 81 thymomas (29 were from patients with MG) using anti-Leu-7, UH-I, and PE-35, each of which is specific for subcapsular-cortical, intra-cortical, and medullary epithelial cells in the normal thymus, respectively, showed 8 immunologic phenotypes. Epithelium of phenotypes U, P, and L is common in the normal thymus, whereas phenotypes UP, UL, PL, UPL, and N are not.

Ring and Addis reported Leu-7–positive epithelium in both the cortico-medullary junction area and cortex, as well as in the subcapsular-cortex in the normal human thymus. Hirokawa et al. reported that medullary epithelium was usually negative for UH-I, but a few positive cells were occasionally observed. Consequently, phenotypes UP, UL, PL, and UPL may be present in the normal thymus as minor subpopulations.

The heterogeneity of normal thymic epithelium, according to the reactivity to monoclonal antibodies specific for different types of keratin polypeptides, reflects different stages in epithelial maturation, i.e., thymic epithelium is capable of undergoing sequential stages of differentiation. Therefore, each epithelial cell of the eight phenotypes may represent different stages of the differentiation in the normal thymus. Recently, some authors suggested that thymic epithelial cells can be derived from common stem cells. Thus, because 14 (17.3%) of the 81 thymomas were predominantly composed of phenotype UPL epithelial cells and because these cells may be present in the normal thymus as a minor subpopulation, we conclude that phenotype UPL epithelium in the normal thymus consists of common stem cells that can differentiate into subcapsular-cortical, intra-cortical, and medullary epithelial cells. These cells may be the target cells for tumorigenesis. Also, we deduced that the epithelial cells of the unusual phenotypes UP, UL, and PL in the normal thymus and in thymoma may represent the intermediate stages of differentiation into epithelium of usual phenotypes.

In our study, 28 (60.9%) of 46 polygonal cell thymomas were of the cortical type and 1 (2.2%) was of the medullary type. Ten (55.6%) of 18 spindle cell thymomas and 7 (41.2%) of 17 mixed cell thymomas were of the medullary type, and 1 (5.6%) of 18 spindle thymomas was of the cortical type. Epithelium in polygonal cell thymoma tends to differentiate into cortical epithelial cells, whereas epithelium in spindle and mixed cell thymomas tends to differentiate into medullary epithelial cells. The unusual phenotype epithelium in each histologic type represents intermediate stages of differentiation into cortical and medullary type epithelial cells.

Morphologically, polygonal, spindle, and mixed cell thymomas appear to correspond to the cortical, medullary, and mixed thymomas, respectively, of Muller-Hermelink et al. Their classification of thymomas is based on the cytologic recognition of different epithelial cells as morphologically similar to normal cortical or medullary epithelial cells. Therefore, the cortical and medullary thymomas of Muller-Hermelink et al. are tumors with epitheliums that tend to differen-

Table 1. Histologic Types and Neoplastic Epithelial Phenotypes

| Phenotypes | Polynomal (%) | Spindle (%) | Mixed (%) | Total (%) |
|------------|---------------|-------------|-----------|-----------|
| U          | 15 (32.6)     | 1 (5.6)     | 0         | 16 (19.8) |
| P          | 1 (2.2)       | 10 (55.6)   | 7 (41.2)  | 18 (22.2) |
| L          | 2 (4.3)       | 0           | 0         | 2 (2.5)   |
| UP         | 3 (6.5)       | 2 (11.1)    | 3 (17.6)  | 8 (9.9)   |
| UL         | 11 (23.9)     | 0           | 0         | 11 (13.6) |
| PL         | 1 (2.2)       | 3 (16.7)    | 4 (23.5)  | 8 (9.9)   |
| UPL        | 11 (23.9)     | 1 (5.6)     | 2 (11.8)  | 14 (17.3) |
| N          | 2 (4.3)       | 1 (5.6)     | 1 (5.9)   | 4 (4.9)   |
| Total      | 46            | 18          | 17        | 81        |

U: UH-I+, PE-35-, Leu-7-, P: UH-I-, PE-35+, Leu-7-; L: UH-I-, PE-35-, Leu-7+; UP: UH-I+, PE-35+, Leu-7-; UL: UH-I+, PE-35-, Leu-7+; PL: UH-I-, PE-35+, Leu-7+; UPL: UH-I+, PE-35+, Leu-7+.

Table 2. Histologic Types and Neoplastic Phenotypes of 29 Thymomas Associated With Myasthenia Gravis

| Histologic types | Polyonal (%) | Spindle (%) | Mixed (%) | Total (%) |
|------------------|--------------|-------------|-----------|-----------|
| U                | 9 (37.5)     | 0           | 0         | 9         |
| P                | 1 (4.2)      | 0           | 2 (40)    | 3         |
| L                | 1 (4.2)      | 0           | 0         | 1         |
| UP               | 0            | 0           | 1 (20)    | 1         |
| UL               | 6 (25.0)     | 0           | 0         | 6         |
| PL               | 1 (4.2)      | 0           | 0         | 1         |
| UPL              | 6 (25.0)     | 0           | 2 (40)    | 8         |
| Total            | 24           | 0           | 5         | 29        |

U: UH-I+, PE-35-, Leu-7-, P: UH-I-, PE-35+, Leu-7-; L: UH-I-, PE-35-, Leu-7+; UP: UH-I+, PE-35+, Leu-7-; UL: UH-I+, PE-35-, Leu-7+; PL: UH-I-, PE-35+, Leu-7+; UPL: UH-I+, PE-35+, Leu-7+.
tiate into cortical and medullary epithelial cells, respectively. However, the mixed thymomas of Muller-Hermelink et al. (the mixed cell thymoma of Rosai and Levine) are not a mixture of cortical type epithelium and medullary type epithelium, but are tumors with characteristics similar to medullary (spindle cell) thymomas that show a radically heterogenous composition. This morphologic heterogeneity can be explained by cells showing a different degree of maturation within the same tumor\(^{20,22,23}\) while exhibiting an immunologically homogenous composition.

We compared the tumor invasiveness of thymomas of usual phenotypes with those with unusual phenotypes. Because the degree of differentiation of unusual phenotype epithelium seemed to be lower than that of usual phenotype epithelium, we thought that the thymoma composed of unusual phenotype epithelial cells would be more invasive; however, no significant difference was detected.

MG was observed in 29 (35.8\%) of 81 cases of thymoma. Histologically, 24 (82.6\%) of 29 thymomas were composed of unusual phenotype epithelial cells. Sixteen (55.2\%) of 29 tumors were composed of the polygonal cell type and the rest were of the mixed cell type. Sixteen (55.2\%) of 29 tumors were composed of cortical epithelial cells, i.e., epithelium of phenotypes U, L, and UL, and 3 (10.3\%) were composed of medullary epithelial cells. The remaining ten tumors were composed of unusual phenotype epithelial cells. Thus, thymomas with MG showed a wide spectrum of immunologic phenotypes. The pathogenesis of MG associated with thymoma is heterogenous and complex.

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