Medical Advances

Epithelial Membrane Antigen Expression in Various WHO Thymoma Types

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Abstract

Background: **Epithelial membrane antigen (EMA) is a glycoprotein, widely used in the differential diagnosis of epithelial neoplasms. Little is known about EMA expression in thymic neoplasms classified based on world health organization (WHO) scheme.**

Material and Methods: **A series of 66 thymic neoplasms were reviewed and classified according to the WHO scheme. These cases along with 7 normal thymic tissues were constructed in three tissue microarray blocks. Slides were stained using anti-EMA monoclonal antibody. Staining patterns were recorded for each tumor, as well as the staining intensity and percentage of stained tumor cells. In case of presence of more than one histologic type, staining was interpreted based on the predominant component.**

Results: **There were 8 type A, 16 type AB, 8 type B1, 5 type B2, 17 type B3 thymomas, and 12 thymic carcinomas. Five staining patterns were recognized, luminal, stromal, stromal & epithelial, cytoplasmic focal and cytoplasmic diffuse in addition to negative. Luminal pattern was seen in cystic component whenever present (all type A cases and 1 type AB). Stromal and stroma & epithelial patterns were seen only in type AB (8 of 16 cases). Types B1 and B2 were negative except for 1 type B2 case (focal). In types B3 and thymic carcinoma, when the staining was present, it was either cytoplasmic diffuse (5 cases each) or cytoplasmic focal (2 cases each). In normal thymus, the staining was restricted to Hassall's corpuscles.**

Conclusions: **EMA expression in thymic neoplasms is variable reflecting the histologic variability of this tumor. Recognizing the various staining patterns of EMA could be a useful tool to subtype thymic neoplasms.**
[N A J Med Sci. 2010;3(3):113-116.]

Key Words: *Thymoma, Thymic carcinoma, WHO schema, EMA*

Received 05/17/2010; Revised 06//21/2010; Accepted 07/01/2010

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Introduction

Thymomas are neoplasms arising from or exhibiting differentiation towards thymic epithelial cells, regardless of the presence and relative numbers of non-neoplastic lymphocytes. The malignant potential is either absent or mild to moderate. Thymomas and thymic carcinomas are uncommon tumors with an annual incidence of approximately 1-5 per million population. They occur at almost all ages with a peak incidence between 55-65 years. Patients exhibit an increased incidence of a second cancer irrespective of the histology of the thymic epithelial tumors. There are two major types of thymoma depending on whether the neoplastic epithelial cells and their nuclei are spindle or oval shaped, and are uniformly bland (type A thymoma) or whether the cells have a predominantly round or polygonal appearance (type B). Type B thymomas are further subdivided on the basis of the extent of the lymphocytic infiltrate and the degree of atypia of the neoplastic epithelial cells into these types, B1 (richest in lymphocytes), B2 and B3 (richest in epithelial cells). Thymomas combining type A with type B1-like or rarely type B2-like features are designated as type AB. Thymic carcinomas on the other hand, have clear cytologic atypia and subdivided according to their differentiation (squamous cell carcinoma, mucoepidermoid carcinoma, etc.).[1]

Epithelial membrane antigen (EMA) is one of several human milk fat globule proteins (HMFGPs) that are derived from the mammary epithelium. The HMFGPs vary greatly in molecular weight (51 kD to >1000 kD). They are predominantly glycoproteinaceous and compos part of the plasmalemma of epithelial cells in areas of the cell membrane overlying tight junctions. In addition, because HMFGPs are packaged in the Glogi apparatus, globular labeling of this structure may be seen immunohistologically.[2] The distribution of HMFGPs is such that many, but not all, non-neoplastic human epithelial cells express at least one member of this proteins family. Exceptions include the gastrointestinal surface epithelium, endocervical epithelium,

prostatic acinar epithelium, epididymis, germ cells, hepatocytes, adrenal cortical cells, rete testes, squamous cells of the epidermis, and thyroid follicular epithelium.[3]

EMA expression in normal thymus and thymic neoplasms has received little attention.[4] Given the fact that immunohistochemistry has become important ancillary tool for tissue diagnosis, we sought to evaluate the expression of EMA in various types of WHO thymomas types and thymic carcinoma.

Material and Methods

Patients and WHO classification

In the period from 1982 through 2009, 66 patients with thymic tumors were seen in hospitals in the Buffalo, New York region. The pathologic features of these patients were retrospectively studied. Cases were re-classified according to the WHO scheme into types A, AB, B1, B2, B3 and thymic carcinoma. Thymic carcinoma cases were subtyped into squamous cell carcinoma and poorly differentiated carcinoma.

Tissue microarray construction and immunohistochemistry

These two procedures were described before.[5-7] For each case, two to seven core samples of tumor tissue were acquired from at least two different donor blocks. A relatively high number of cores were taken when variable histologic features existed in one case.

Slides were stained with anti-EMA antibody (clone E29, Dako, dilution 1/600, with no pretreatment) with positive and negative controls (full colonic section, epithelium positive and muscularis propria negative). Sections were cut at 5μm, placed on charged slides and dried in a 60^0C oven for 1 hour. Upon return to room temperature, the slides were in three changes of xylene and rehydrated using graded alcohols. Endogenous peroxidase activity was quenched with aqueous 3% hydrogen peroxide for 15 minutes and washed with phosphate buffered saline with 0.05% Tween-20 (PBS-T). Antigen retrieval was then performed. After a PBS-T wash, casein 0.03% (in PBS-T) was used as a block for 30 minutes and then the primary antibody was applied to the slides and left for 30-60 minutes. A PBS-T wash was followed by the biotinylated secondary antibody for 30 minutes. The PBS-T was followed by the streptavidin complex for 30 minutes. PBS-T was used as a wash and the Chromagen 3,3'-diaminobenzidine (DAKO, Carpenteria, CA) was applied for 5 minutes (the color reaction product was brown). The slides were counterstained with Hematoxylin.

The cases were scored semiquantitatively incorporating staining intensity and percentage of positive cells. The staining intensity was recorded as 0 (negative, complete absence of staining), 1 (weak, staining that barely seen), 2 (moderate, staining between weak and strong) and 3 (strong, clear homogenous intense staining). The final score was the sum total of the product of the staining intensity (0 to 3) and the percentage of stained cells within the tumor. A score greater than 30 was required for the results to be recorded as positive expression. The pattern of staining (luminal, stromal, stromal & epithelial, cytoplasmic focal and cytoplasmic diffuse) was also recorded.

Statistical Analysis

Statistical analyses for comparing groups in regards to categorical variables were performed using Fisher's exact test. Statistical analysis was performed using the Statistical Analysis System version 9.1.3 (SAS Institute Inc. North Carolina). A nominal significance level of 0.05 was used.

Results

Pathologic data

There were 8 type A, 16 type AB, 8 type B1, deparaffinized 5 type B2, 17 type B3 thymomas, and 12 thymic carcinomas. There were 6 cases of squamous cell carcinoma (1 moderately differentiated and 5 poorly differentiated) and 6 cases of poorly differentiated carcinoma.

Table 1. EMA staining results with staining pattern according to WHO types.

		Staining pattern				
WHO type (N.)	**Positive**	**Luminal**	**Stromal**	**Stromal & epithelial**	**Cytoplasmic focal**	**Cytoplasmic diffuse**
A (8)	8(100)*	8(100)	0(0)	0(0)	0(0)	0(0)
AB (16)	9(56.3)	1(11.1)	6(66.7)	2(22.2)	0(0)	0(0)
B1 (8)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
B2 (5)	1(20)	0(0)	0(0)	0(0)	1(100)	0(0)
B3 (17)	7(41.2)	0(0)	0(0)	0(0)	2(28.6)	5(71.4)
Carcinoma (12)	7(58.3)	0(0)	0(0)	0(0)	2(28.6)	5(71.4)
Total (66)	32(48.9)	9(28.1)	6(18.8)	2(6.2)	5(15.6)	10(31.3)

*Number (%)

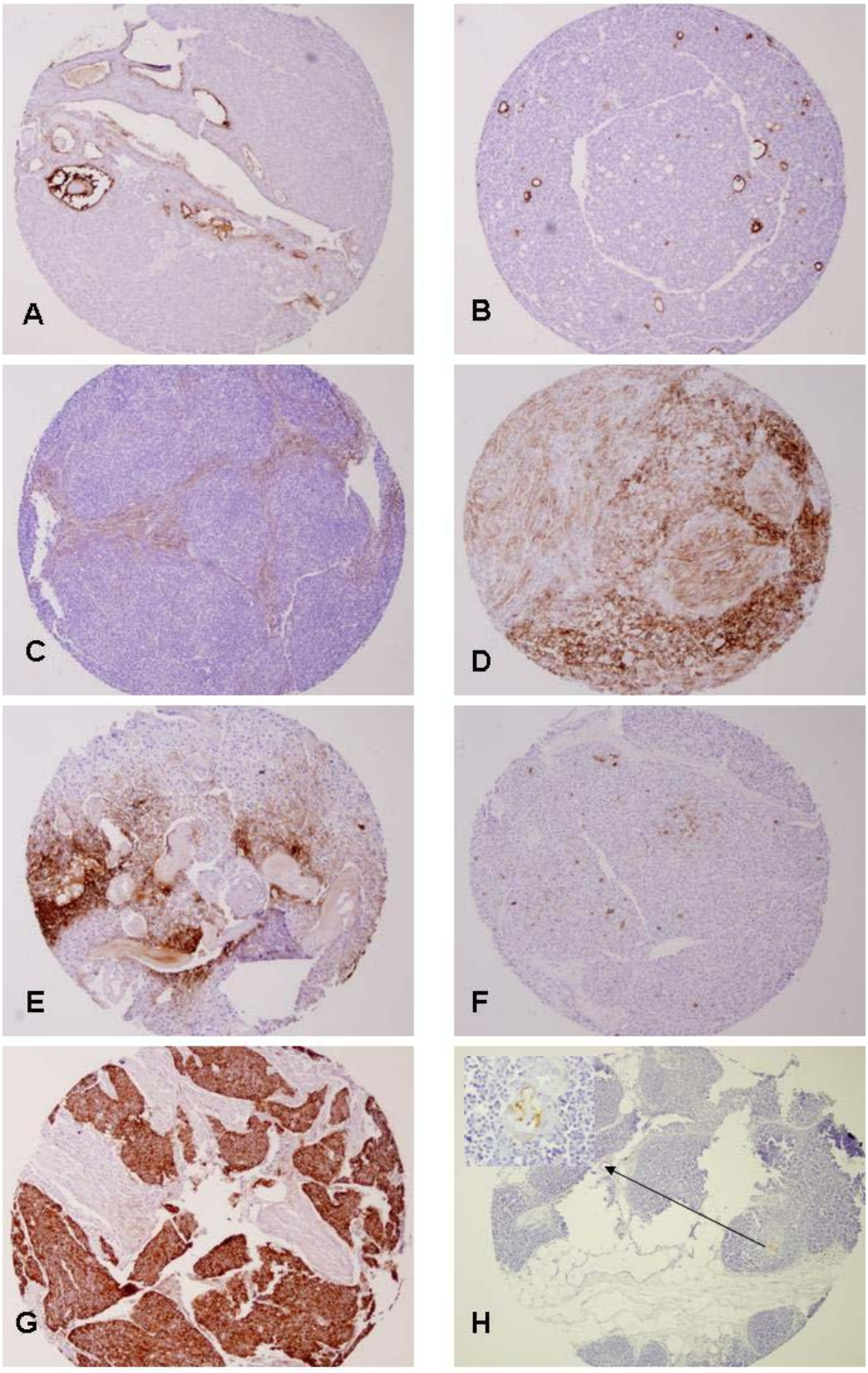

Figure A-H.
Various staining patterns inn thymoma WHO types (10x each);
A, microcystic luminal staining in type A;
B, macrocystic luminal staining in type A;
C, spindle stromal staining in type AB;
D, spindle medullary and epithelial cortical in type AB;
E, cytoplasmic diffuse staining pattern in type B3;
F, cytoplasmic focal staining pattern in type B3;
G, cytoplasmic diffuse staining in thymic carcinoma;
H, normal thymus showing staining of Hassal's corpuscle (arrow and inst).

EMA expression in various WHO types

Overall, 32 of 66 (48.9%) cases stained positive with EMA (**Table 1**). Five different staining patterns were recognized, luminal, stromal, stromal & epithelial, cytoplasmic focal and cytoplasmic diffuse. Luminal staining pattern was restricted to cases where cystic structures is present, large (**Figure 1A**) or small (**Figure 1B**). All type A cases showed luminal staining, while one of 16 type AB showed this type of

staining. Stromal and stromal & epithelial staining patterns were restricted to type AB (**Figures 1C** and 1D, respectively). Types B1 and B2 did not show any staining, except for one type B2 where the staining was focal. Cytoplasmic diffuse or focal staining patterns were features of types B3 (**Figures 1E** and **1F**, respectively) and thymic carcinoma (**Figure 1G**). However, it was noted that thymic carcinoma had more diffuse staining than type B3 (**Figures 1E** and **1G**). Interestingly, normal thymuses were negative except for Hassall's corpuscles (**Figure 1H** and inset).

Using Fisher's exact test and comparing the groups in regards to categorical variables, there was significant difference in staining of luminal pattern for type A versus type AB or all types combined (p value=0.005 and <0.0001). Stromal or stromal & epithelial staining patterns were only seen in type AB comparing with the rest the tumors (p<0.0001). Given the small number of type B2 (n=5), statistical analysis could not be performed. However, combining types B1 and B2, negative staining was almost restricted to these two types (p=0.02). EMA was not useful to differentiate type B3 (7 of 17 cases) from thymic carcinoma (7 of 12 cases).

Discussion

Thymic neoplasms are rare tumors. Recognizing thymoma with its WHO subtypes is usually straightforward. However, in a small needle biopsy setting and given its rarity particularly in a small community hospital, recognizing thymomas could be challenging. We have outlined different staining patterns of EMA in various WHO types of thymic neoplasms. This variability reflects the histologic complexity of thymomas. Although there was no statistical difference in the overall staining (positive vs. negative) with regard to tumor type except for negative staining for types B1 and B2 combined, using the outlined staining patterns could be of help in reaching the correct WHO subtype. We found that luminal staining pattern is indicative of type A, stromal or stromal & epithelial are indicative of type AB, while cytoplasmic focal or diffuse staining patterns are indicative of type B3 and thymic carcinoma.

The distribution of EMA in thymic neoplasms has been previously reporte.[4] However, this was before the WHO classification. Applying what has been reported to the current WHO subtypes of thymic neoplasms could be confusing. Therefore, this study provides a clearer approach to the EMA staining patterns in thymic neoplasms with the WHO subtypes.

It is unclear why the staining varies with the type of the tumor. EMA composes part of the plasmalemma of epithelial cells in areas of the cell membrane overlying tight junctions.[6] This may explain the luminal staining pattern in type A where the luminal membrane is made of combined luminal membranes of the medullary cells. The variability in staining distribution between type B3 and thymic carcinoma was explained by Fukai et al. They postulated that it could be related to the degree of tumor differentiation.[4] The increase of EMA expression after malignant neoplastic transformation is suggested to be related to poor intercellular contact, which may help to sustain the unrestricted growth characteristic of neoplasms.[8] Why types B1 and B2 rarely express EMA is also unclear. However, we found that in normal thymus, only Hassall's corpuscles expressed EMA but not the cortical cells. Since types B1 and B2 are of cortical origin, it is consistent with biologic expression of this marker. Type B3, on the other hand is also of cortical cell origin, but expresses EMA. Type B3 tumor is a unique entity, as it behaves with moderate malignant potential comparing with the other two ends, tumors with low malignant potential (types A, AB, B1 and B2) and overtly malignant tumors (thymic carcinoma). The fact that EMA expression in terms of its presence and distribution falls between types B1/B2 (negative) and thymic carcinoma (more diffuse) is consistent with the previous explanation of gradual transformation to malignant neoplasms with poor intercellular contact. It was described that negative EMA is a good marker to differentiate type B2 from type B3.[1]

In summary, recognizing the various EMA expression types in thymic neoplasms could be a useful tool for the pathologist to subclassify thymic tumors according to WHO scheme. However, the number of cases studied is small. Therefore, these results should be interpreted with caution and a larger cohort is needed to validate these results.

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