

E-Cadherin Expression in Thymomas

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For the purpose of investigating the pattern of E-cadherin (E-CD) expression in thymomas, 72 cases were immunostained using monoclonal antibody (HECD-1) and microwave-enhanced immunohistochemical method on formalin-fixed, paraffin-embedded tissue sections. The thymomas were classified according to modified Müller-Hermelink classification. The spindle-shaped, medullary type tumor epithelial cells in medullary (3 cases) and composite type (20 cases) thymomas rarely expressed E-CD except in focal areas showing microcystic change observed in 8 cases. Meanwhile, the cohesive epithelioid tumor cells in every case of well-differentiated thymic carcinomas (WDTC) (29 cases) expressed E-CD. The epithelial cells in cortical type (13 cases) expressed stronger E-CD compared with those of organoid type (7 cases). In cases of WDTC, admixed with cortical type, we observed increasing expression of E-CD as the tumor epithelium forms cohesive sheets. We could not find any loss of E-CD expression in invasive foci of the 11 cases of high-staged WDTC examined. Since the results of our study show a strong correlation between E-CD expression and epithelioid morphology of the tumor, E-CD seems to play a major role as a morpho-regulatory factor rather than as a suppressor of invasion in organotypic thymomas.

Key Words: E-cadherin, thymomas, immunohistochemistry

Thymomas are primary epithelial neoplasms derived from cortical and/or medullary epithelium of the thymus. Among several clinicopathologic parameters associated with prognosis, tumor invasiveness expressed by stage has been regarded as the most important prognostic factor (Masaoka *et al.* 1981; Verley and Hollmann, 1985; Lewis *et al.* 1987). Several recent reports using a newly-developed his-

togenetic classification of thymomas by Müller-Hermelink instead of the traditional histologic classification based on the proportion of lymphocytes and epithelial cells demonstrated that well-differentiated thymic carcinoma (WDTC) and cortical-type thymomas behaved more aggressively compared to the other types (Marino and Müller-Hermelink, 1985; Kirchner and Müller-Hermelink, 1989; Pescarmona *et al.* 1990; Quintanilla-Martinez *et al.* 1993).

Cadherins are a group of adhesion molecules mediating cell-cell adhesion mainly by homotypic interactions. Among these, E-cadherin (E-CD) is one of the best characterized and it is confined to all epithelia regardless of their embryonal origin. Recently, there has been increasing evidence that decreased expression of E-CD may be an important mechanism of invasion and metastasis of carcinomas (Takeichi, 1991; Gumbiner, 1996; Jiang, 1996).

The principal aim of this study was to analyze the pattern of E-CD expression in organotypic thymo-

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mas to determine whether altered expression of E-CD may be of relevance to the different biologic behavior of thymomas according to the histologic subtype.

MATERIALS AND METHODS

Patients

Seventy-two cases of thymomas from patients operated on between 1980 and 1995, in which adequate paraffin blocks and clinical history were available, were retrieved from the files of the Department of Pathology, Yonsei University College of Medicine and Yonsei Wonju University College of Medicine. Patients included 31 females and 41 males, and their ages ranged from 14 to 73 years (mean 48 years). Three cases of grossly unremarkable thymic tissue obtained during cardiac and lung surgeries were used for the immunohistochemical staining of normal thymus.

Tumour classification and staging

Based on the modified classification system of Müller-Hermelink, utilizing the criteria outlined by Quintanilla-Martinez *et al.* (1993), 72 cases of thymomas were classified as medullary type, composite type, organoid type, cortical type, and WDTC. The patients were staged according to the classification of Masaoka *et al.* (1981).

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissue sections (5 μ thick) were heated at 60°C for 1 hour. The slides were then deparaffinized and hydrated through xylene and graded alcohols, and placed in distilled water. To block endogenous peroxidase activity, they were incubated for 10 minutes in 3% H₂O₂. The sections were microwaved (750 W) for 10 minutes in sodium citrate buffer (pH 6.0, 0.01 M) after washing in distilled water. After cooling for 20 minutes, they were incubated for 20 minutes in normal human serum (1:10) followed by overnight incubation in a refrigerator with the monoclonal anti-E-cadherin antibody HECD-1 (5 μ g/ml; Zymed, San Francisco, CA, U.S.A.). The sections were then rinsed and incubated for 30 minutes with biotinylated anti-mouse immunoglobulin and streptavidin conjugated peroxidase (LSAB kit, Dako Corporation, Carpinteria, CA, U.S.A.). The peroxidase reaction was developed with 3-amino-9-ethylcarbazide. Negative controls were carried out by substituting primary antibody with mouse non-immune serum.

RESULTS

Tumour classification and staging

The most common histologic subtype was WDTC (29 cases) followed by 20 cases of composite type, 13 cases of cortical type, 7 cases of organoid type, and 3 cases of medullary type. All patients with medullary, composite, and organoid types, except one case of stage IV organoid type, were stage I or

Tabel 1. Types of thymomas versus stages

Histologic types	Stage I	Stage II	Stage III	Stage IV	Total
Medullary	2	1	0	0	3
Composite	15	5	0	0	20
Organoid	4	2	0	1	7
Cortical	7	3	3	0	13
WDTC	10	8	11	0	29
Total	38	19	14		72

WDTC: well-differentiated thymic carcinoma

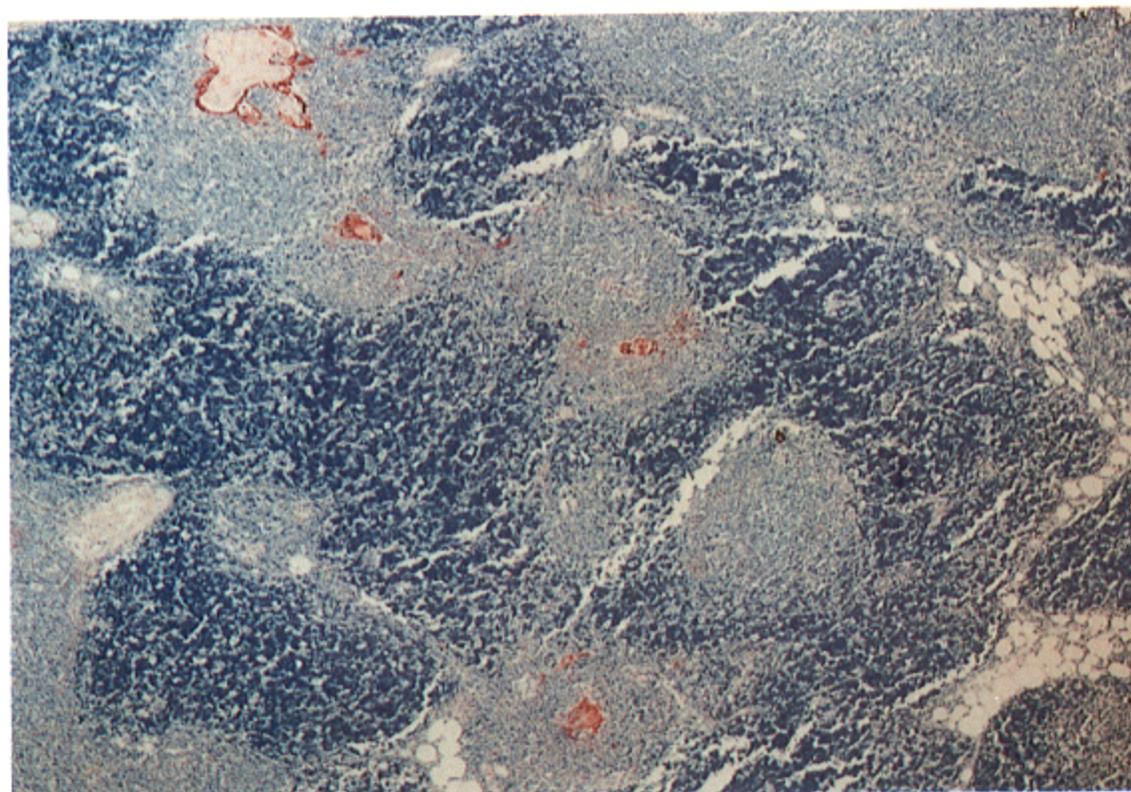


Fig. 1. Normal thymus showing strong cytoplasmic expression of E-CD in Hassall's corpuscle.

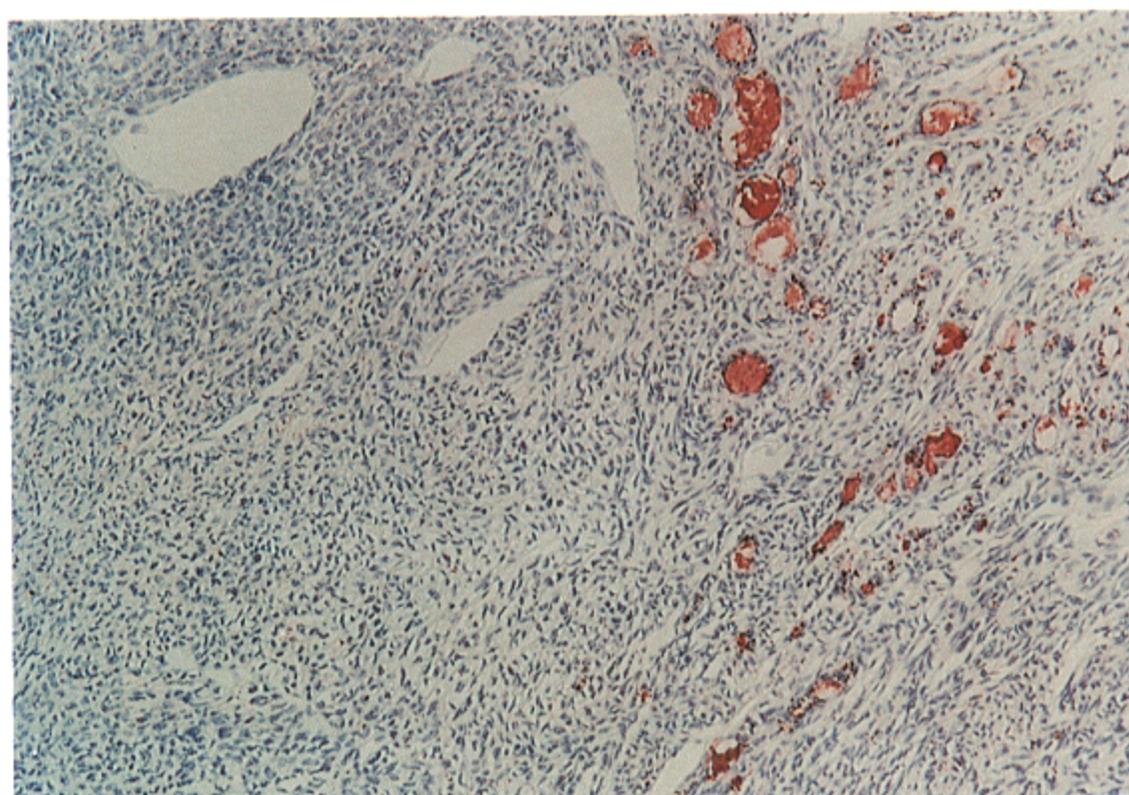


Fig. 2. Composite thymoma showing intraluminal and cytoplasmic E-CD expression only in area with microcystic change.

stage II, while 3 of 13 cases of cortical type and 11 of 29 cases of WDTC were stage III (Table 1).

E-CD expression pattern in normal thymus

In the normal thymus, the most striking feature was strong cytoplasmic expression of E-CD in Hassall's corpuscles (Fig. 1). A few scattered epithelial cells, mainly in medullary areas, expressed weak

E-CD. No other cells in the thymus except thymic epithelial cells expressed E-CD.

E-CD expression pattern according to the histologic types of thymomas Medullary and composite type of thymomas

The spindle shaped medullary type tumor epithelial cells observed in 23 cases of medullary and

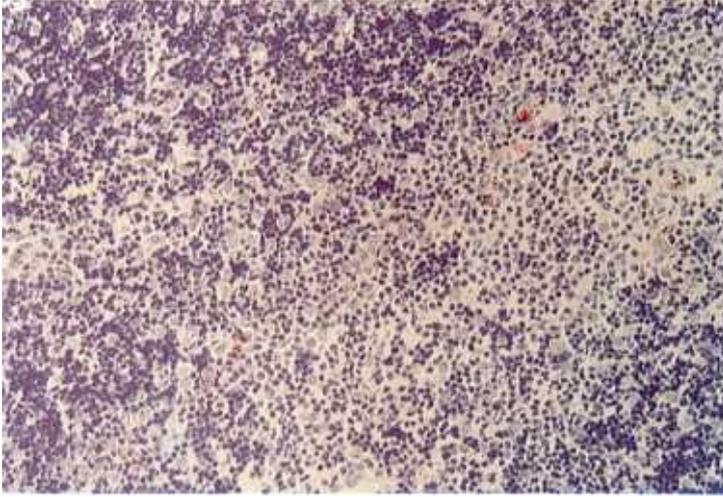


Fig. 3. Organoid thymoma showing scattered cells expressing E-CD in medullary area.

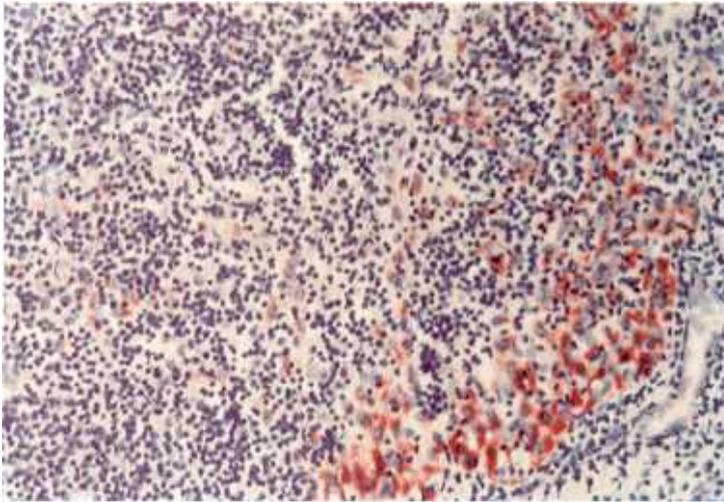


Fig. 4. Cortical thymoma showing strong intracytoplasmic E-CD expression within tumor epithelial cells on the periphery of the lobules with polarity of positive reaction.

composite thymomas rarely expressed E-CD except in areas showing microcystic change, which was observed in 8 cases. In these areas, we observed a strong expression of E-CD even in the secreted materials in the lumen as well as the cytoplasmic granular reactions in the lining epithelium (Fig. 2). Areas rich in thymocytes within composite type thymomas demonstrated rare, scattered epithelial cells expressing weak E-CD.

Organoid type thymomas

The expression pattern of E-CD was very similar to that of the normal thymus in that some epithelial cells, mainly in the medullary areas, expressed weak E-CD (Fig. 3).

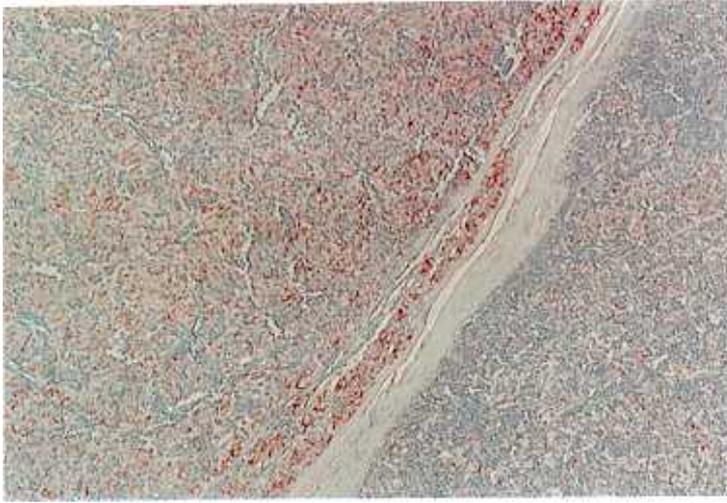


Fig. 5. Well-differentiated thymic carcinoma possessing areas of cortical thymoma showing stronger expression of E-CD in solid epithelial tumor area.

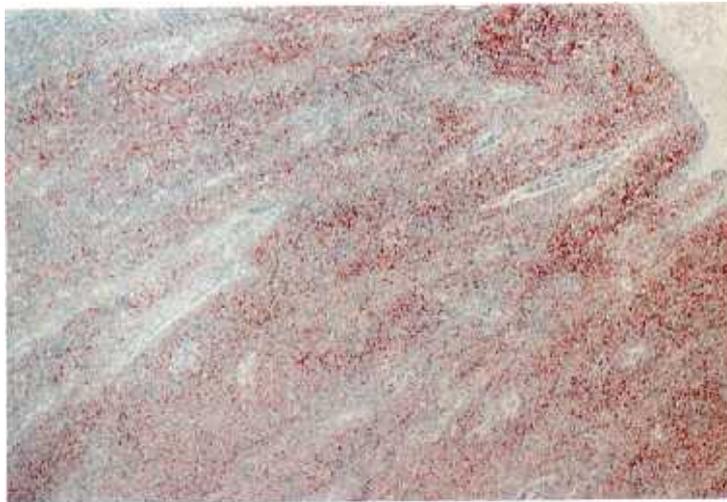


Fig. 6. Well-differentiated thymic carcinoma showing diffuse membranous expression of E-CD.

Cortical type thymomas

The tumor epithelial cells in cortical type thymomas were generally larger than those of organoid type thymomas and therefore looked more epithelioid. Four out of 13 cases of cortical type thymomas expressed strong granular cytoplasmic and/or cell membrane pattern E-CD and we observed the

polarity of the positive reaction in some tumor cells. The expression of E-CD was more prominent along the periphery of the tumor nests, showing some palisading arrangement of tumor cells (Fig. 4). In cases of thymomas having both cortical type thymoma and WDTC, there was a definite tendency of stronger expression of E-CD as tumor cells formed sheets (Fig. 5). There were no differences of E-CD

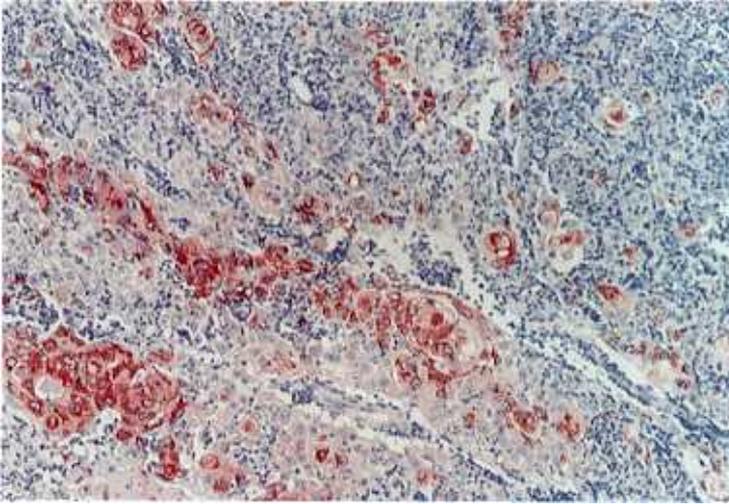


Fig. 7. Well-differentiated thymic carcinoma with more distinct squamoid differentiation showing strong intracytoplasmic expression of E-CD as in Hassall's corpuscles.

expression among low and high stages of cortical thymomas at presentation.

Well-differentiated thymic carcinomas

Every case of the WDTC showed diffuse, cell-membrane pattern expression of E-CD (Fig. 6) although some heterogeneity of staining within the tumor mass was noted. The case of WDTC showing more prominent squamoid differentiation exhibited stronger cytoplasmic E-CD expression (Fig. 7) compared to the case showing some spindling of the tumor cells. We could not find any difference of E-CD expression between the primary tumor and the invasion foci in the lungs of 11 cases examined.

DISCUSSION

WDTC and cortical type thymoma, defined by the histogenetic classification of thymomas, are known to behave more aggressively compared to other types of thymomas (Marino and Müller-Hermelink, 1985; Kirchner and Müller-Hermelink, 1989; Pescarmona *et al.* 1990; Kirchner *et al.* 1992; Pescarmona *et al.* 1992; Quintanilla-Martinez *et al.* 1993). To explain their different biologic behaviors, several studies on

the phenotypes (Marino and Müller-Hermelink, 1985; Kirchner and Müller-Hermelink, 1989; Quintanilla-Martinez *et al.* 1993), DNA contents and kinetics (Rahilly *et al.* 1991; Kuo and Lo, 1993; Pich *et al.* 1995; Yang *et al.* 1996) and oncoprotein expression (Brocheriou *et al.* 1995; Hayashi *et al.* 1995; Tateyama *et al.* 1995; Chen *et al.* 1996) of the tumor cells have been done using various methods. However, no studies have so far clearly demonstrated reasons to explain their different biologic behavior.

Cell adhesion molecules are molecules which are involved in cell-cell and cell-matrix adhesions and they include members of cadherin, integrin, immunoglobulin, selectin, and proteoglycan superfamilies. They are key mediators for the assembly of individual cells into three-dimensional tissue, and therefore are involved widely in morphogenesis and embryogenesis. Cadherins are transmembrane Ca^{2+} dependent adhesion molecules required for the maintenance of solid tissue and E-CD, mainly expressed in epithelial tissues, which have been the most studied and best characterized cadherins. In addition to its roles in embryogenesis and morphogenesis of normal tissue, E-CD is thought to act as an important suppressor of epithelial tumor-cell invasion and metastasis based on the results of many *in vivo* and *in vitro* studies (Behrens *et al.* 1989; Takeichi, 1991; Birchmeir *et al.* 1993; Sorscher *et al.* 1995; Gabbert

et al. 1996; Gumbiner, 1996; Jiang, 1996).

In this study, we analyzed the expression pattern of E-CD in thymomas using an immunohistochemical method. As with other monoclonal antibodies, monoclonal antibody to E-CD was initially applied only to fresh, frozen tissue but recent studies using highly sensitive immunohistochemical methods showed convincing results even on routinely processed, paraffin-embedded tissue sections (Sorscher *et al.* 1995; Cowley and Smith, 1996; Gabbert *et al.* 1996). The results of our study revealed that the expression of E-CD is correlated with epithelioid phenotype tumors in terms of cytologic and architectural features. Among thymomas, the spindle cells of medullary and composite subtypes showed only focal expression of E-CD mainly confined to areas showing microcystic changes, while the epithelioid cells of cortical subtype and WDTC expressed E-CD strongly. In cases of WDTC mixed with cortical type thymoma, there was a definite increase of E-CD expression as the tumor cells formed a solid mass. The epithelial cells in organoid thymomas showing neither prominent epithelioid cytologic features nor a compact, solid mass of tumor cells rarely expressed E-CD. The pattern of E-CD expression in organoid thymoma exactly recapitulated that of normal thymus, further supporting that "organoid" is a better term than "predominantly cortical" for this type of thymoma maintaining well-organized normal structures even after tumor formation (Pescarmona *et al.* 1991).

There have been several studies on the immunophenotype of the neoplastic epithelial cell as well as the accompanying thymocytes in thymomas (Marino and Müller-Hermelink, 1985; Sato *et al.* 1986; Kirchner and Müller-Hermelink, 1989; Quintanilla-Martinez *et al.* 1993; Chan *et al.* 1995). The results of most of these studies could not define specific immunophenotypes of the tumor epithelium in different tumor types, while the immunophenotypes of the accompanying thymocytes matched well with the tumor subtypes. Our data strongly indicates that the spindle tumor epithelial cells in medullary and composite type thymomas are less mature cells compared to the polygonal tumor epithelial cells in cortical type thymoma and WDTC in terms of epithelial differentiation. This suggestion could be supported by the results of the previous *in vitro*

studies demonstrating carcinoma cell lines showing strong E-CD expression have a more epithelioid appearance, while those without E-CD expression have a more spindled appearance (Behrens *et al.* 1989; Birchmeir *et al.* 1993). The expression of cadherin in anaplastic large cell lymphoma (Ashton-Key *et al.* 1996) and epithelioid peripheral nerve sheath tumor (Smith *et al.* 1994), both possessing abundant cytoplasm, as well as in epithelioid type A melanocytes, but not in spindle type C melanocytes (Cowley and Smith, 1996), also supports the validity of our idea. The localization of E-CD expression only in the areas showing the microcystic change in cases of medullary and composite thymomas also suggests that it is not a degenerative process but an active process accompanied by E-CD expression. Considering that the spindle cell variant of WDTC could mimic medullary thymoma, a distinctly different pattern of E-CD expression between WDTC and medullary thymoma might be used as a diagnostic aid due to the fact that our data demonstrated only the spindle cells in WDTC showed diffuse membranous staining of E-CD.

The strong expression of E-CD in biologically-aggressive subtypes of organotypic thymomas, the lack of correlation between E-CD expression and the stages, no loss of E-CD in invasive foci of WDTC, and a strong correlation between E-CD expression and epithelioid morphology of the tumor cells could be interpreted as indicating E-CD does not act as a suppressor of invasion, but as a morphoregulatory factor in organotypic thymic epithelial tumors.

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