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Thymoma: tumour type related to expression of epidermal growth factor (EGF), EGF-receptor, p53, v-*erb* B and *ras* p21

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Abstract As clinicopathological features may not be sufficient to predict the progression of thymoma, we have carried out what we believe to be the first immunohistochemical study describing the relationship between the different types of thymoma and the tumour stage, on the one hand, and the expression of epidermal growth factor (EGF), EGF-receptor (EGFR), p53, v-*erb* B and *ras* p21, on the other. The positive rates versus histological types and Masaoka's clinical stages in the 47 cases were as follows: p53 (non-invasive thymoma: 41.7%; malignant thymoma category I: 82.4%; malignant thymoma category II: 83.3%), EGF (non-invasive thymoma: 4.2%; malignant thymoma category I: 11.8%; malignant thymoma category II: 33.3%) and EGFR (non-invasive thymoma: 8.3%; malignant thymoma category I: 35.3%; malignant thymoma category II: 66.7%); p53 (stages I and II: 51.7%; stages III and IV: 77.8%), EGF (stages I and II: 3.4%; stages III and IV: 22.2%) and EGFR (stages I and II: 13.8%; stages III and IV: 44.4%). These data suggest that p53 may be implicated in the initial stages of tumorigenesis and that increased expression of EGF and EGFR may play a role in thymoma progression.

Key words Thymoma · Epidermal growth factor-receptor · p53 · v-*erb* B · *ras* p21

Introduction

The traditional histological classification of thymoma was described by Bernatz et al. [5] and Lattes [27] and further evaluated by Lewis et al. [29], distinguishing between predominantly lymphocytic, predominantly epithelial, mixed, and predominantly spindle cell type thymomas. Levine and Rosai also distinguish between non-invasive (benign) thymomas and invasive thymic epithelial tumours; the latter are further separated into malignant thymomas (category I) and invasive thymic epithelial tumours with cytological atypia (malignant thymomas, category II, also called thymic carcinomas). The histogenetic classification was recently characterized by Müller-Hermelink and co-workers, classifying thymomas according to the morphological and functional relationship between neoplastic thymic epithelial cells and their normal counterparts in the thymus, and distinguishing among medullary, mixed, predominantly cortical and cortical thymomas, and well differentiated thymic carcinomas [22]. The histological classification of Bernartz and Lattes is of no prognostic significance; however, the classification of Levine and Rosai is highly relevant with respect to prognosis, but it usually needs the total tumour specimen to determine whether or not invasion is present. We have classified our tumours according to this classification. Finally, the histogenetic classification ("Müller-Hermelink system") allows a better prediction of invasiveness and metastatic potential on the basis of histology alone [25, 40]. We have applied the traditional clinicopathological classification of thymoma according to Levine and Rosai, and another classification system ("Müller-Hermelinksystem") [22, 31].

Many clinicopathological studies of thymoma tumorigenesis and development have been reported [25, 40], but few have examined what factors rule its biological actions [23, 35, 37]. To find possible factors affecting transformation and progression of thymoma, we have carried out an immunohistochemical study to examine the relationship between histological types and clinical stages of thymoma [32] and expression of epidermal growth factor (EGF), EGF-receptor (EGFR), p53, v-*erb* B and *ras* p21.

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Table 1 Clinical and histological features of a series of the thymomas classified according to Levine and Rosai classification (*MG* myasthenia gravis, *M-H* Müller-Hermelink, *EGF* epidermal growth factor, *EGFR* EGF receptor, *P* predominantly, *WDTC* well differentiated thymic carcinoma, *SCC* squamous cell carcinoma, *LEC* lymphoepithelioma-like-carcinoma, *Undiff Ca* undifferentiated carcinoma)

Case	Age	Sex	Stages[a]	MG	Clinical and histopathologic diagnosis	M-H system	Immunohistochemical findings[b]				
							p53	*ras* p21	v-*erb* B	EGF	EGFR
1	58	Female	I	–	Non-invasive thymoma (lymphocytic)	Cortical	–	–	–	–	–
2	64	Female	I	–	Non-invasive thymoma (lymphocytic)	Cortical	–	–	–	–	–
3	68	Female	I	–	Non-invasive thymoma (lymphocytic)	Cortical	–	–	–	–	–
4	48	Male	I	–	Non-invasive thymoma (lymphocytic)	Cortical	+(58)	–	–	–	–
5	56	Male	I	+	Non-invasive thymoma (lymphocytic)	Cortical	+(50)	–	–	–	–
6	45	Female	I	+	Non-invasive thymoma (lymphocytic)	P Cortical	+(62)	–	+(81)	–	–
7	40	Female	I	–	Non-invasive thymoma (lymphocytic)	Mixed	–	–	–	–	–
8	40	Male	I	–	Non-invasive thymoma (lymphocytic)	Mixed	–	–	–	–	–
9	45	Male	I	–	Non-invasive thymoma (lymphocytic)	Mixed	–	–	–	–	–
10	60	Male	I	–	Non-invasive thymoma (mixed type)	Cortical	+(73)	+(78)	–	–	–
11	50	Male	I	–	Non-invasive thymoma (mixed type)	Cortical	+(57)	–	–	–	–
12	23	Male	I	–	Non-invasive thymoma (mixed type)	Cortical	–	–	–	–	–
13	40	Female	I	+	Non-invasive thymoma (mixed type)	P Cortical	–	–	++(89)	±	+(81)
14	38	Male	I	–	Non-invasive thymoma (mixed type)	P Cortical	+(53)	–	+(78)	+(84)	–
15	56	Female	I	–	Non-invasive thymoma (mixed type)	Mixed	+(58)	–	–	–	–
16	56	Female	I	–	Non-invasive thymoma (mixed type)	Mixed	–	–	–	–	–
17	56	Male	I	–	Non-invasive thymoma (mixed type)	Mixed	–	–	–	–	–
18	45	Male	I	–	Non-invasive thymoma (epithelial)	Cortical	+(63)	–	±	–	–
19	40	Male	I	–	Non-invasive thymoma (epithelial)	P Cortical	+(61)	–	–	–	–
20	53	Female	I	–	Non-invasive thymoma (epithelial)	P Cortical	–	–	–	–	–
21	31	Male	I	+	Non-invasive thymoma (epithelial)	WDTC	–	–	+(95)	–	+(99)
22	54	Male	I	–	Non-invasive thymoma (spindle cell)	Medullary	–	–	–	–	–
23	43	Female	I	–	Non-invasive thymoma (spindle cell)	Medullary	–	+(92)	+(99)	±	–
24	63	Male	I	–	Non-invasive thymoma (spindle cell)	Medullary	+(55)	–	–	–	–
25	68	Male	IVa	–	Malignant thymoma, category I (mixed type)	WDTC	+(56)	+(89)	+(87)	+(83)	±
26	61	Female	II	–	Malignant thymoma, category I (mixed type)	WDTC	+(62)	+(78)	+(77)	–	+(81)
27	52	Male	III	+	Malignant thymoma, category I (mixed type)	WDTC	+(79)	±	+(81)	–	–
28	45	Female	II	+	Malignant thymoma, category I (mixed type)	Cortical	+(64)	–	+(79)	±	++(76)
29	46	Female	II	+	Malignant thymoma, category I (mixed type)	Cortical	+(67)	+(79)	±	–	–
30	33	Female	IVa	+	Malignant thymoma, category I (mixed type)	P Cortical	–	–	–	–	–
31	60	Male	II	–	Malignant thymoma, category I (mixed type)	P Cortical	+(55)	+(75)	–	–	–
32	57	Female	II	–	Malignant thymoma, category I (mixed type)	Mixed	+(59)	–	–	–	–
33	51	Female	IVa	+	Malignant thymoma, category I (mixed type)	Mixed	+(87)	–	±	±	+(84)
34	67	Male	IVa	–	Malignant thymoma, category I (mixed type)	Mixed	–	–	–	–	–
35	58	Female	III	–	Malignant thymoma, category I (epithelial)	Cortical	+(59)	–	+(82)	–	–
36	69	Female	III	–	Malignant thymoma, category I (epithelial)	WDTC	+(87)	–	+(99)	+(94)	+(91)
37	54	Male	III	–	Malignant thymoma, category I (epithelial)	WDTC	+(55)	±	–	–	–
38	55	Female	IVb	–	Malignant thymoma, category I (epithelial)	WDTC	+(84)	+(89)	±	–	+(99)
39	42	Male	III	+	Malignant thymoma, category I (epithelial)	WDTC	+(53)	±	–	–	–
40	62	Female	IVa	–	Malignant thymoma, category I (epithelial)	Mixed	–	+(84)	++(76)	–	±
41	42	Female	III	+	Malignant thymoma, category I (epithelial)	WDTC	+(58)	±	±	–	+(90)
42	69	Male	III	–	Malignant thymoma, category II (SCC)		+(72)	–	±	+(99)	+(99)
43	63	Male	III	–	Malignant thymoma, category II (SCC)		+(89)	–	+(97)	+(92)	+(95)
44	79	Male	III	–	Malignant thymoma, category II (SCC)		+(77)	+(89)	±	±	+(90)
45	62	Male	IVb	+	Malignant thymoma, category II (SCC)		+(92)	+(95)	±	±	+(99)
46	58	Female	IVb	–	Malignant thymoma, category II (LEC)		+(56)	–	–	–	–
47	81	Male	IVa	–	Malignant thymoma, category II (undiff ca)		–	+(99)	–	–	–

[a] Masaoka's clinical stages

[b] Immunohistochemical findings: intensity of reactivity was graded on a scale of – to ++, numbers in parentheses: % positive cells/total epithelial cells in each lesion

Materials and methods

Tissues from 47 cases of thymoma (24 non-invasive thymoma, 17 malignant thymoma category I, and 6 malignant thymoma category II) were routinely fixed with 10% buffered formalin and embedded in paraffin blocks. Four-micron-thick sections cut from the blocks were stained immunohistochemically for growth factors (EGF and EGFR) and other agents (p53, v-*erb* B and *ras* p21). The avidin-biotin complex technique was employed on all specimens. For immunohistochemical analysis, normal thymic tissue was used as normal control. According to the criteria of Masaoka [32], each tumour was clinically staged on the basis of surgical data and histological examination of the specimen as follows: stage I, no capsular invasion (24 cages); stage II, capsular or pleural invasion (5 cases); stage III, invasion into a neighbouring organ such as pericardium, great vessels, or lung (9 cases); stage IVa, pleural or pericardial dissemination (6 cases); stage IVb, lymphogenous or hematogenous metastasis (3 cases; Table 1). The histological types of each clinicopathologic stage of thymoma classified by the Müller-Hermalink system are also listed in Table 1.

In pre-experimental studies, both frozen and paraffin-embedded sections were stained immunohistochemically with appropriate reagents and under stable conditions. The antibodies used in this study were anti-EGF, anti-EGFR, anti-p53, anti-v-*erb* B, and anti-*ras* p21. The anti-EGF monoclonal antibody (mAb) was obtained from the ascites fluid of Balb/c mice injected with a hybridoma prepared by the fusion of mouse myeloma cell (p3UI) with spleen cells from a Balb/c mouse immunized with recombinant hEGF (Wakunaga, Japan; dilution 1:100) [48]. The anti-EGFR mAb (referred to a number 528) was raised against the EGFR on A431 cells derived from an epidermoid carcinoma of the vulva (Oncogene Science, New York; dilution 1:20) [21, 33]. The anti-p53 mAb (referred to as BP53-12) was the product of mouse hybridomas cloned from a polyethylene glycol-induced fusion of splenocytes from a Balb/c mouse hyperimmunized with recombinant human wild type p53 protein [4] which reacts with wild and mutant forms of p53 products (Japan Tanner Corporation, Japan; dilution 1:10). The v-*erb* B oncogene product polyclonal antibody (pAb) was raised against a bacterially expressed *erb* B DNA restriction fragment (*Bam*HI/*Bam*HI); the antiserum immunoprecipitates the *erb* B protein from avian erythroblastosis virus (AEV)-transformed chicken fibroblasts and recognizes the EGFR protein (Medac Molecular Biology; dilution 1:400) [15]. The NCC-RAS-001, anti-*ras* oncogene product p21 antibody, kindly supplied by Dr. S. Hirohashi (National Cancer Centre Research Institute, Japan), was raised against recombinant p21 containing valine substituted for glycine at position 12 [19]; it reacts with both normal p21 and point-mutated p21 (dilutions 1:500 and 1:1000) [24, 36].

For immunohistochemistry sections were deparaffinized with xylene and routinely dehydrated through a series of graded alcohols. Prior to EGF, EGFR, and *ras* p21 staining, histological sections were digested with a 0.05% trypsin solution for 10 min at 37° C, treated with a methanol solution containing 3% hydrogen peroxide (H_2O_2) for 5 min to eliminate endogenous peroxidase, washed in TRIS buffer for 5 min, incubated for 5 min at room temperature in normal goat serum, washed in TRIS buffer for 5 min, and incubated at room temperature in primary antibodies: p53 and EGFR overnight, and EGF, v-*erb* B, and *ras* p21 for 30 min. After two 5 min TRIS buffer washes, the sections were incubated in biotinylated anti-mouse immunoglobulin at room temperature: EGFR and p53 for 60 min, and EGF and *ras* p21 for 30 min. The v-*erb* B sections were incubated in biotinylated anti-rabbit immunoglobulin for 30 min, then washed twice in TRIS buffer for 5 min each. Reactions with the avidin-biotin peroxidase complex (DAKO Laboratories) were carried out at room temperature: EGFR and p53 for 60 min, and EGF, v-*erb* B, and *ras* p21 for 30 min, then the sections were rinsed with TRIS buffer for 15 min. After colour development with 0.03% diaminobenzidine (including 0.01% H_2O_2), the sections were counterstained with Mayer's haematoxylin or methyl green for 30 s. As negative control, the primary antibody was replaced with phosphate-buffered saline. Slides were examined under a light microscope, and the intensity of immunostaining was graded on a scale of –, negative; ±, trace; +, positive; ++, strongly positive. Antibody reactivity was quantified by evaluating at least five histological sections or at least 200 epithelial cells, then the percentage of epithelial cells reacting with a given antibody was determined.

Results

Histological and clinicopathological features of 47 cases (25 male and 22 female, ranging in age between 23 and 81 years) of non-invasive and malignant thymoma are shown in Table 1. Immunohistochemical studies of EGF, EGFR, p53, v-*erb* B and *ras* p21 in normal thymic tissues demonstrated that thymic lymphocytes were totally negative, and epithelial cells (other than keratinizing cells in medullary Hassall's corpuscles, which were sometimes faintly stained) were also negative. Since the positive staining of the keratinizing cells in Hassall's corpuscles may have been due to non-specific antibody binding to keratinizing cells, the staining of non-keratinizing cells was used as normal control. The EGF, EGFR, v-*erb* B, and *ras* p21 stained diffusely in the cytoplasm of neoplastic epithelial cells, but the cells negative for these antibodies did not stain at all. Some cells positive for EGFR, v-*erb* B and *ras* p21 stained more strongly on the cellular membranes. We interpreted the results of p53 as positive when the nuclei were stained. In all cases positive for EGF, EGFR, v-*erb* B and *ras* p21, over three-quarters of epithelial cells were stained; in all cases positive for p53, over half of epithelial cells' nuclei were stained. The positive rates of p53, *ras* p21, v-*erb* B, EGF and EGFR in the 47 cases are shown in Table 2. EGF which was expressed more strongly in thymic carcinoma (malignant thymoma category II) than in cases of non-invasive thymoma or in malignant thymoma category I, stained positive in one non-invasive thymoma (4.2%), in two malignant thymomas category I (11.8%) and in two malignant thymomas category II (33.3%; Fig. 2). *ras* p21 stained positive in two cases of non-invasive thymoma (8.3%), in six cases of malignant thymoma category I (35.3%) and in three cases of malignant thymoma category II (50%) (Fig. 5).

Table 2 Frequency of expression of p53, *ras* p21, v-*erb* B, EGF and EGFR in different types of thymomas according to Levine and Rosai classification (*MT* malignant thymoma)

	p53	*ras* p21	v-*erb* B	EGF	EGFR
Non-invasive thymoma	10/24	2/24	5/24	1/24	2/24
Lymphocytic	3/9	0/9	1/9	0/9	0/9
Mixed type	4/8	1/8	2/8	1/8	1/8
Epithelial	2/4	0/4	2/4	0/4	1/4
Spindle cell	1/3	1/3	0/3	0/3	0/3
MT category I	14/17	6/17	7/17	2/17	6/17
Lymphocytic	0/0	0/0	0/0	0/0	0/0
Mixed type	8/10	4/10	4/10	1/10	3/10
Epithelial	6/7	2/7	3/7	1/7	3/7
MT category II	5/6	3/6	1/6	2/6	4/6
SCC	4/4	2/4	1/4	2/4	4/4
LEC	1/1	0/1	0/1	0/1	0/1
Undiff Ca	0/1	1/1	0/1	0/1	0/1

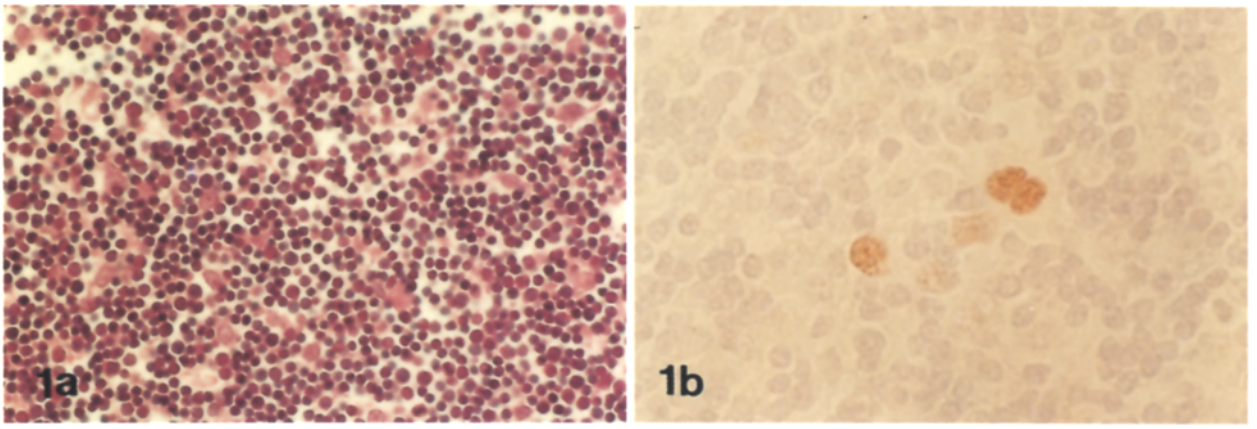

Fig. 1a, b Non-invasive thymoma, lymphocytic predominant type, case number 4. **a** Haematoxylin and eosin (H&E), ×171.4. **b** A few epithelial cells stained positively for p53, ×514

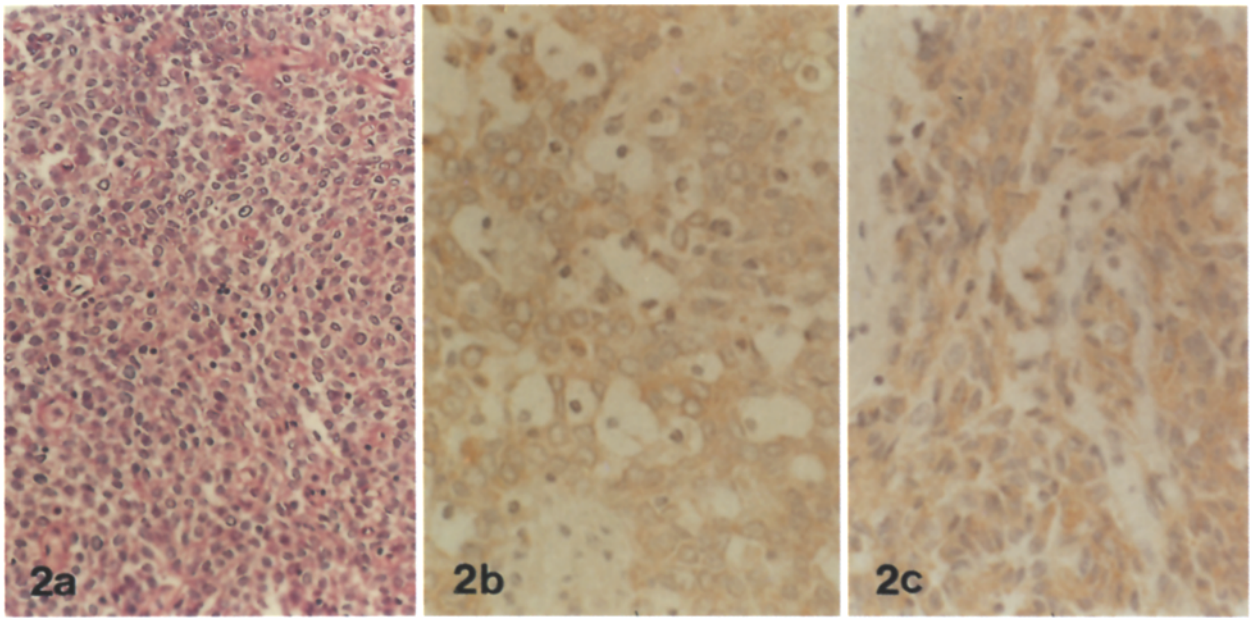

Fig. 2a–c Malignant thymoma, category I, epithelial predominant type, case number 36. **a** H&E, ×85.7. **b** Epithelial cells diffusely stained in the cytoplasm for epidermal growth factor (EGF), ×343. **c** Epithelial cells diffusely stained in the cytoplasm for EGF receptor (EGFR), ×343

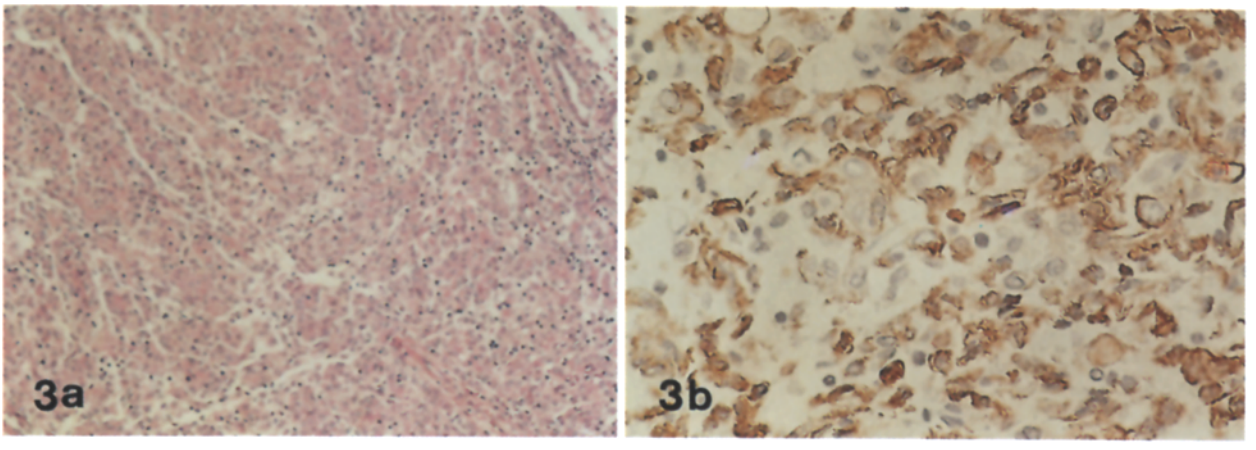

Fig. 3a, b Malignant thymoma, category I, epithelial predominant type, case number 40. **a** H&E, ×85.7. **b** Epithelial cells stained strongly for v-*erb* B, ×343

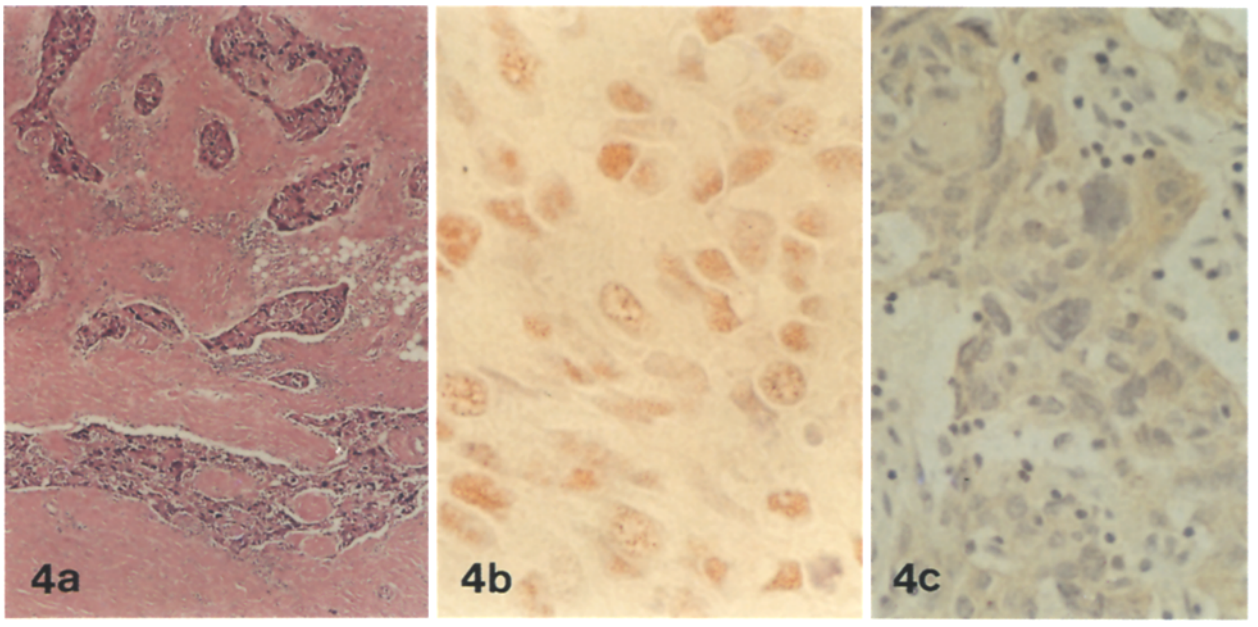

Fig. 4a–c Malignant thymoma, category II, squamous cell carcinoma, case number 45. **a** H&E, ×85.7. **b** The nuclei of cancer cells stained positively for p53, ×514. **c** Cancer cells diffusely stained for EGFR, ×514

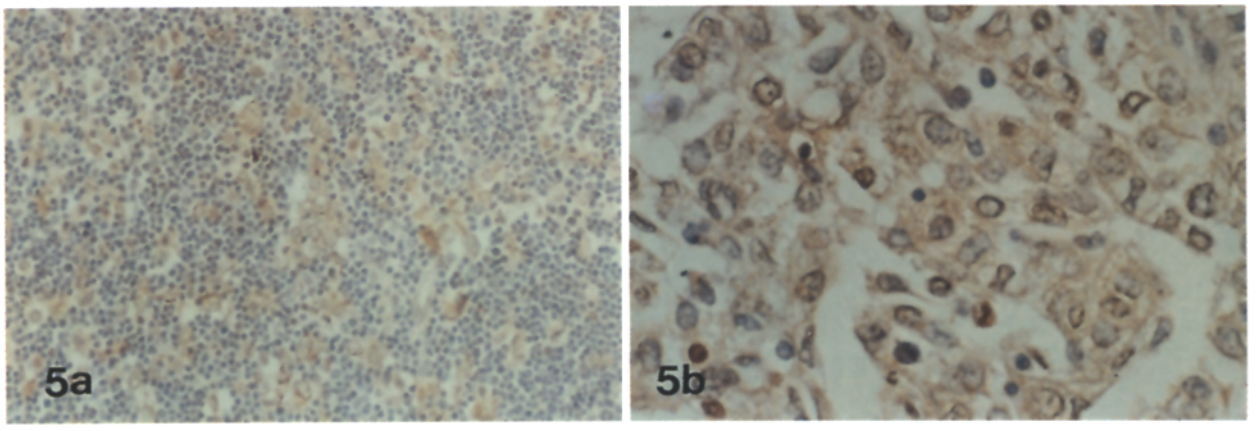

Fig. 5a, b Non-invasive thymoma, mixed type, case number 10 and malignant thymoma, category II, squamous cell carcinoma, case number 44. **a** Many epithelial cells stained positively for *ras* p21, case number 10, ×171.4. **b** Most carcinoma cells positive far *ras* p21, case number 44, ×343

v-*erb* B stained positive in five cases of non-invasive thymoma (20.8%), in seven cases of malignant thymoma category I (41.2%) and in one case of malignant thymoma category II (16.7%; Fig. 3). EGFR stained positive in two cases of non-invasive thymoma (8.3%), in six cases of malignant thymoma category I (35.3%) and in four cases of malignant thymoma category II (66.7%; Figs. 2, 4). Overexpression of the p53 protein appeared in many cases of non-invasive and malignant thymomas (Figs. 1, 4). The p53 product stained positive in ten cases of non-invasive thymoma (41.7%), 14 cases of malignant thymoma category I (82.4%) and five cases of malignant thymoma category II (83.3%).

Clinical stage versus the positive rates of p53, *ras* p21, v-*erb* B, EGF and EGFR in the 47 cases were as follows: stage I: 24 cases (p53: 10 cases; *ras* p21: 2 cases; v-*erb* B: 5 cases; EGF: 1 case; EGFR: 2 cases); stage II: 5 cases (p53: 5 cases; *ras* p21: 3 cases; v-*erb* B: 2 cases; EGF: none; EGFR: 2 cases); stage III: 9 cases (p53: 9 cases; *ras* p21: 1 case; v-*erb* B: 4 cases; EGF: 3 cases; EGFR: 5 cases); stage IV: 9 cases (p53: 5 cases; *ras* p21: 5 cases; v-*erb* B: 2 cases; EGF: 1 case; EGFR: 3 cases; Table 1).

Discussion

Thymomas are neoplasms arising from thymic epithelial cells with a variable number of associated non-neoplastic lymphoid cells [28, 29]. In some types of thymomas, the ratio of the number of neoplastic epithelial cells to non-neoplastic lymphoid cells is too small to detect gene mu-

Table 3 Frequency of expression of p53, *ras* p21, v-*erb* B, EGF and EGFR in different types of thymomas according to Müller-Hermelink system

	p53	*ras* p21	v-*erb* B	EGF	EGFR
Medullary	1/3	1/3	1/3	0/3	0/3
Mixed	3/10	1/10	1/10	0/10	1/10
P cortical	4/7	1/7	3/7	1/7	1/7
Cortical	8/12	2/12	2/12	0/12	1/12
WDTC	8/9	3/9	5/9	2/9	5/9

tation, even by dot-blot hybridization [12]. Immunostaining is a useful tool for detecting these aberrations [1, 6, 36, 44]. Histopathological examination of thymoma often fails to predict its malignant potential because the morphology of malignant thymoma category I does not significantly differ from that of non-invasive thymoma. We examined the histopathological and clinicopathological diagnoses of 47 cases of non-invasive and malignant thymoma according to the classification of Levine and Rosai [28]: non-invasive thymoma: 51.06%; malignant thymoma category I: 36.17%; and malignant thymoma category II: 12.77%. The histological type of malignant thymoma category II [46] is shown in Table 1. We had only six cases of malignant thymoma category II, and the incidence of 12.77% was lower than that of 25.3% reported by Kuo et al. [26]; this deviation may be due to geographical differences and remains to be explored [25]. To find new and significant predictors of invasive/metastatic thymomas, we characterized these three subgroups of thymoma by histopathological and clinicopathological features and by immunostaining with antibodies to EGF, EGFR, p53, v-*erb* B, and *ras* products.

Overexpression of p53 protein in its mutated, long-life form appears in primary carcinoma of the lung [16], breast carcinoma [2, 3, 7], basal cell carcinoma of the human skin [43], oesophageal carcinoma [17, 41], and gastric carcinoma [17]. Some investigators have reported that *p53* and Ki-*ras* genetic alterations are found in up to 50% of cases of colorectal carcinoma [8].

In our and others' previous studies, overexpression of the p53 protein appeared at advanced stages of tumorigenesis in the colon and the liver [14, 38] while it showed positivity at relatively earlier stages in nasopharyngeal neoplasms (Hayashi et al., unpublished data). In the present work, overexpression of the p53 protein appeared in many cases of non-invasive thymoma and malignant thymoma (non-invasive thymoma: 41.7%; malignant thymoma category I: 82.4%; malignant thymoma category II: 83.3%) indicating that p53 is implicated in the initial stages of the tumorigenesis of thymoma.

EGF is a polypeptide (molecular weight, 6,045) consisting of 53 amino acids [10], that is distributed in the submandibular gland, parotid gland, and duodenal Brunner gland, stomach, pancreas, and bone marrow [20], and that promotes growth and differentiation, not only in epithelial, but also in mesenchymal cells [13]. Expression of EGF and EGFR can be associated with an adverse prognosis in some tumours: gastric carcinomas expressing both EGF and EGFR simultaneously tend to be infiltrative and invade deeper into the stomach wall [45]; EGFR is always more or less expressed in undifferentiated squamous cell carcinoma (SCC), but the staining decreases in the moderate to well differentiated SCC of the oral mucosa [9]. The EGF system may play a role in the growth regulation of cancer cells, and the expression of EGFR may be associated with more biological aggressiveness in laryngeal carcinoma [42]. In this work, EGF and EGFR were expressed more strongly in malignant thymoma category II (EGF: 33.3%; EGFR: 66.7%) than in non-invasive thymoma (EGF: 4.2%; EGFR: 8.3%) or in malignant thymoma category I (EGF: 11.8%; EGFR: 35.3%). These results suggest that the invasive nature of thymoma may be related to the expression of EGF and EGFR. The v-*erb* B2, which relates to EGFR but represents a truncated EGFR whose extracellular part is lost [15], and which may accelerate cell cycles without ligands (such as EGF and TGF α), stained positively in non-invasive thymoma (20.8%) as well as in malignant thymoma category I (41.2%) and malignant thymoma category II (16.7%). It therefore seems to play no significant role in the progression of thymoma.

The expression of the Ki-, N- and Ha-*ras* genes has been found in benign and malignant tumours [11, 30, 34, 39, 47]. Mukai et al. [36] described the probable role of *ras* oncogene product, p21 protein, in oncogenesis and/or progression of thymoma; their description corresponds with our results.

According to the histological classification of "Müller-Hermelink system", all cases of the medullary thymoma (spindle cell epithelial thymoma by the Levine and Rosai classification) belong to Masaoka's clinical stage I, while the other histological types are more or less of invasive nature (Table 1); this suggests that all thymomas (other than those of purely spindle or medullary cell types) have the potential for invasion and aggressive behaviour [25, 40]. Our immunohistochemical study of the positive rate versus histological type according to the "Müller-Hermelink system" revealed that all cases of medullary thymoma (only three cases of medullary thymoma were available) were negative for EGF and EGFR (Table 3). Despite its smallness, this number of cases may be related to the rarity of invasiveness of medullary thymoma. However, well differentiated thymic carcinoma manifested a higher frequency of p53, *ras* p21, v-*erb* B and EGFR expression; this corresponds more to malignant thymoma category II (thymic carcinoma) than to other histological types and may well reflect its histological nature as reported by Pescarmona [40].

Ishii [18] described the relationship between Masaoka's clinical stages and the prognosis of thymoma patients after complete resection in 26 tumours of stages I and II, and successful resection of 18 patients ot stages III and IV. The 5- and 10-year survival rates, respectively, were as follows: 92% and 92% (stage I), 100% and 100% (stage II), 82% and 63% (stage III), and 32% and 16% (stage IV). In our study, clinical stages versus the positive rates of the antigens are shown above. These da-

ta indicate that enhanced expression of EGF (stages I and II: 3.4%; stages III and IV: 22.2%) and EGFR (stages I and II: 13.8%; stages III and IV: 44.4%) may predict an adverse prognosis in thymoma.

In summary, the results of our study suggest that the mutation of p53 probably plays a role in the initial stages of tumorigenesis, and that expression of EGF and EGFR may be implicated in the progression of thymoma.

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