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p53 Alterations in thymic epithelial tumours

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Abstract The prognosis of thymic epithelial tumours depends on malignant behaviour that cannot always be predicted on histological grounds. This study aimed at identifying a molecular marker that would be useful in overcoming the drawbacks of histopathology. Forty-four thymic epithelial tumours were analysed for alterations of the tumour suppressor gene p53 using immunohistochemistry (antibodies D0-1 and CM-1) and PCR-based single-strand conformation polymorphism and DNA sequencing. Histological and clinical evaluation and also p53 analysis revealed three major tumour groups: non-organotypic thymic carcinomas with frequent p53 alterations (7/9) and occurrence of p53 gene mutations (2/9); malignant thymomas with frequent p53 alterations but without p53 gene mutations (11/18); and benign thymomas with rare p53 alterations and without p53 gene mutations (2/17). In non-organotypic thymic carcinomas p53 was detected with both antibodies. In contrast, thymomas lacked immunoreaction with D0-1 suggesting alteration of the antibody-binding site. Overall immunohistochemical results correlated with clinical stages ($P < 0.01$), pathohistology ($P < 0.01$), and survival times ($P < 0.05$). We consider immunohistochemical p53 detection to be a useful new prognostic factor for the evaluation of thymic epithelial tumours.

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Introduction

The thymus is the site of histologically very different types of tumours owing to the heterogeneity of its cellular composition. Thymic epithelial proliferations are subdivided into organotypic thymomas and non-organotypic thymic carcinomas [1, 2]. Thymic epithelial tumours occur mainly in the middle aged and elderly and are the most common pathological lesions of the superior anterior mediastinum [3]. Whereas non-organotypic thymic carcinomas are highly malignant tumours, thymomas may be benign or malignant and are generally slow-growing. There are two competitive histopathological classifications of thymic epithelial tumours, both of which claim to be prognostically relevant. The revised classification of Rosai and Levine [2, 4] focuses on tumour invasiveness and matches the clinical need for tumour staging perfectly. The capsular invasion is judged according to Masaoka's staging [5]. The revised Rosai classification defines three tumour entities: non-invasive thymomas (Masaoka stages I and II), invasive/metastatic thymomas (Masoaka stages III and IV), and thymic carcinomas (Masaoka stages III and IV). Whereas non-invasive and invasive/metastatic thymomas are cytologically unremarkable, thymic carcinomas show typical features of malignancy. Although the Rosai and Levine classification includes some histocytological criteria, these are not considered to be informative about clinical behaviour, except for thymic carcinomas. Marino, Müller-Hermelink, and Kirchner (MMHK) introduced a pathohistological classification of thymic epithelial tumours that involves the use of histogenetic criteria to define two major entities: organotypic thymomas and non-organotypic thymic carcinomas [6–8]. In this classification thymomas are further subdivided into benign medullary and mixed-type thymomas, malignant predominantly cortical and cortical thymomas, and well-differentiated thymic carci-

Table 1 Primer sequences for the amplification of exons 5–8 of the p53 gene (intronic primer pairs [20]: *right* 3′ → 5′, *left* 5′ → 3′, *bp* base pairs, *Tm* melting temperatures used for calculation of the appropriate annealing temperature)

Primer	Exon (bp)	Tm (°C)	Sequence	PCR product (bp)
5 right	5 (184)	54,6	5′-CTGGAGAGACGACAGGGCTG-3′	247
5 left		45,1	5′-TTCAACTCTGTCTCCTTCCT-3′	
6 right	6 (113)	56,0	5′-GGGAGGAGGGGTTAAGGGTG-3′	204
6 left		64,1	5′-GGTCTCCCCAAGGCGCACTGG-3′	
7 right	7 (110)	65,1	5′-AGGCTGGGGCACAGCAGGCC-3′	208
7 left		64,4	5′-GGTCTCCCCAAGGCGCACTGG-3′	
8 right	8 (137)	71,0	5′-GCGAATTCTCCACCGCCTCTTGTCCTGC-3′	223
8 left		63,8	5′-GCGAATTCATTTCCTTACTGCCTCTTGC-3′	

nomas. Several clinical retrospective studies have shown a correlation between MMHK tumour types and clinical behaviour: medullary and mixed-type thymomas are mainly non-invasive tumours (Masoaka stages I and II) with a good prognosis. Predominantly cortical and cortical thymomas, well-differentiated thymic carcinomas and non-organotypic thymic carcinomas tend to be invasive/metastatic tumours (Masaoka stages III and IV) with a poor prognosis with regard to relapse and survival [9–11]. The MMHK classification was recently confirmed in a larger trial [12], leaving less uncertainty as to whether a thymoma is malignant or not. However, the histogenetic origin of thymoma subtypes from medullary or cortical epithelial cells of the normal thymus remains uncertain. For this reason the MMHK classification is not generally accepted.

Invasiveness continues to be the only generally accepted hallmark of growth behaviour and prognosis. It is critical to note that invasiveness is a late event in tumour progression. There is a need for additional findings that could help to identify malignant thymomas before advanced tumour progression and irrespective of the invasion status. Mutations of the p53 gene and p53 immunoreactivity, respectively, are promising markers of tumour progression and prognosis, as alterations have been shown to be involved in carcinogenesis and in the prognosis of the majority of human malignancies [13]. In many tumours p53 alterations are early events in oncogenesis [13, 14] and can be detected by well-established techniques, including immunohistochemistry and polymerase chain reaction (PCR) based methods. In this study thymic epithelial tumours were analysed for p53 alterations to test the validity of the pathological MMHK classification and to address its value in the prognostic evaluation of these tumours independently of tumour stage.

Materials and methods

The samples stemmed from patients who had undergone tumour resection between 1979 and 1991, 42 of whom were seen at postoperative follow-up. There were 17 male and 25 female patients. The median age was 46.5 years. We classed 17 tumours as stage I, 9 as stage II, 5 as stage III, and 11 as stage IV (Masaoka's staging system). The median overall survival time was 79.9 months after surgery.

Table 2 Cycle programmes for the amplification of exons 5–8 of the p53 gene. For the amplification of DNA extracted from formalin-fixed, paraffin-embedded tissue extended cycle numbers were necessary. The amplification of exon 8 included an automated prolongation of the extension time for 1 s per cycle. This helped to overcome the decreasing activity of the Taq-polymerase in this 45-cycle assay

Exon	Denaturation	Annealing	Extension	Cycles
5	92° C 1 min	58° C 30 s	72° C 30 s	40
6	92° C 1 min	60° C 30 s	72° C 30 s	40
7	92° C 1 min	67° C 30 s	72° C 30 s	40
8	92° C 1 min	65° C 30 s	72° C 30 s → 74 s	45

Forty-four formalin-fixed (pH 7.5), paraffin-embedded samples of thymic epithelial tumours were stained by routine procedures (H&E, Giemsa, Gomori silver stain) and typed according to the up-dated MMHK histopathological classification [8]. The tumour samples consisted of 3 medullary thymomas, 14 mixed-type thymomas, 4 predominantly cortical thymomas, 7 cortical thymomas, 7 well-differentiated thymic carcinomas, and 9 non-organotypic thymic carcinomas (1 sarcomatoid, 1 neuroendocrine, 2 lymphoepithelial, 1 squamous, 3 thymic carcinomas that were not further classifiable, 1 undifferentiated carcinoma). Whole-blood-derived DNA was available from 15 patients.

Sections (3 μm thick) were taken from representative tumour blocks in all 44 cases, dewaxed (xylene, ethanol) and boiled in a microwave oven in saturated lead thiocyanate solution (2x 5 min, 870 W) for antigen recovery. Immunohistochemistry was carried out with overnight incubation (4 °C) of the anti-p53 monoclonal antibody D0-1 [15] or the polyclonal antibody CM-1 [16] together with the conjugated double APAAP method using new fuchsin as a chromogen [17]. Both antibodies were kindly provided by D.P. Lane, Dundee. Reactions were considered to be informative when at least 10% of epithelial cells showed intranuclear signals regardless of signal intensity [18]. In critical cases p53-cytokeratin (CK1/3) double staining was performed to determine the cell type with p53 immunoreactivity (data not shown).

For DNA extraction sections (7 μm) were again taken from the paraffin blocks previously used in all 44 cases for immunohistochemistry; they were transferred to microfuge tubes, dewaxed (xylene, ethanol), and subjected to protein digestion (proteinase K) overnight [19]. A 1:10 dilution of each lysate in sterile water was used as a template for PCR. Whole-blood DNA was extracted by cell lysis and subsequent DNA precipitation.

The phylogenetically conserved exons of the p53 gene (exons 5–8) that are the main mutation sites in human cancers [13] were amplified by PCR with intronic primer pairs [20] (Table 1) using a sealing-wax-based hot-start technique. For specificity and sensitiv-

ity enhancement the reaction volume was subdivided into two parts (25 µl each). Part 1 consisted of 8 µl sterile water, 8 µl dNTPs (200 mM dATP, dTTP, dCTP, dGTP), 5 µl 10 × reaction buffer (100 mM Tris-HCl, 500 mM KCl, 10 mM $MgCl_2$, 0,01 % w/v gelatin, pH 8.3 [exons 5–7], pH 9.2 [exon 8], Perkin-Elmer), and 4 µl diluted template DNA. Part 2 consisted of 22.5 µl sterile water, 1 µl of each primer (0.1 mM [exons 5–7], 0.2 mM [exon 8]), and 0.5 µl Taq polymerase (5 U/µl, Perkin-Elmer). Part 1 was transferred to a thermal block cycler (TC-1, Perkin-Elmer) for DNA denaturation (4 min, 94°C), and part 2 was added during a supplementary high-temperature period (2 min, 92°C). After completion, the exon-specific cycling program was started (Table 2).

The sensitivity of the SSCP assay was tested for by diluting DNA derived from a mutated cell line (HTB 38 HT-29, Fa. ATTCC; point mutation in exon 8 of the p53 gene) with lymphocyte-derived DNA from a healthy donor. A 5% mutated DNA sample gave rise to a band shift pattern (data not shown).

Prior to electrophoresis 2.5 µl of PCR products of 42 cases were diluted with 10 µl denaturation buffer (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanole), heat-denaturated for 10 min (95°C), and shock-cooled. SSCP analysis was carried out for 8–13 h at constant power (8 W) with a concentration and ionic gradient (DISK), using a horizontal TGGE water-cooled electrophoresis chamber device (diagen). The 20×20 cm slab polyacrylamide (PAA) gels contained of 2% glycerol, 10×TPE, TEMED, 10% APS, 5% PAA (stacking gel) or 15 % PAA (separating gel). The running buffer consisted of 10×TBE. DNA fragments were silver-stained according to standard protocols [19].

PCR-products of 3 cases with reproducible band shifts in SSCP analysis were subjected to a double-stranded sequencing reaction. The reaction was carried out using fluorescence labelled nucleotides and an oscillation detection device (373 DNA sequencer, Applied Biosystems). Sequencing reactions were done commercially (Sequiserve, Vaterstetten, Germany).

Results

Intranuclear immunoreactivity was observed in epithelial cells but not in thymocytes or stromal cells (Fig. 1). Immunoreaction with the polyclonal antibody CM-1 was observed in 5 out of 7 well-differentiated thymic carcinomas (Fig. 1), 4 out of 7 cortical thymomas, 2 out of 4 predominantly cortical thymomas, 2 out of 14 mixed-type thymomas, but none of the medullary thymomas. Staining intensity was variable. In contrast, immunoreactivity with the monoclonal antibody D0-1 was not observed in these tumour groups. Among non-organotypic thymic carcinomas 7 out of 9 were reactive with the polyclonal antibody CM-1 and among these, 4 with the monoclonal antibody D0-1. Staining was uniformly intense with both antibodies (Fig. 5a, c).

Correlation of immunohistochemical results with histological tumour types, clinical stages, and the occurrence of myasthenia gravis was carried out using Fisher's

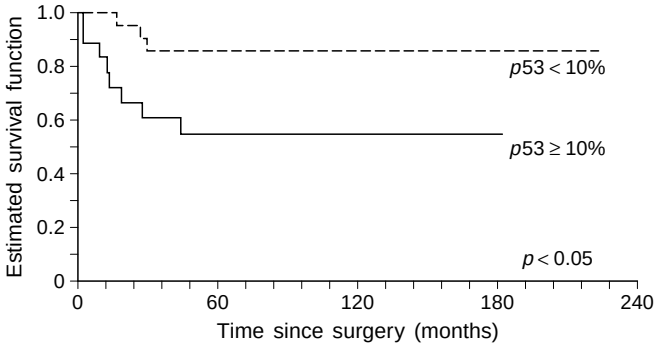

Fig. 2 Kaplan-Meier survival curve estimates of patients with detectable p53 protein in their thymic epithelial tumours (*continuous line*) and of patients without detectable p53 protein in their thymic epithelial tumours (*broken line*). There is a significant correlation between survival curve estimates: patients whose tumours showed detectable p53 protein had a substantially worse survival than those with tumours that did not exhibit p53 protein. ($P < 0.05$, log-rank test)

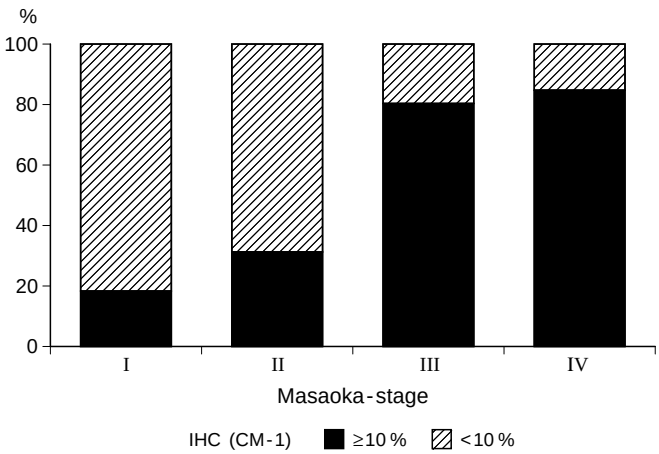

Fig. 3 Bar chart showing the distribution of immunohistochemical detection of p53 with the antibody CM-1 in relation to clinical stages (Masaoka's system). *Black portion* of each bar represents immunoreactive tumours; *hatched portion* represents non-reactive tumours. *I-IV* are the clinical stages: *I* non-invasive intracapsular tumour growth; *II* microscopic transcapsular tumour invasion of surrounding fat tissue; *III* gross tumour invasion of adjacent organs; *IV* tumours with pleural/pericardial dissemination and/or metastasis. There is a significant correlation between immunohistochemical p53 detection and clinical stages: tumours with detectable p53 protein are mostly invasive (stages III and IV) ($P < 0.01$, Fisher's exact test)

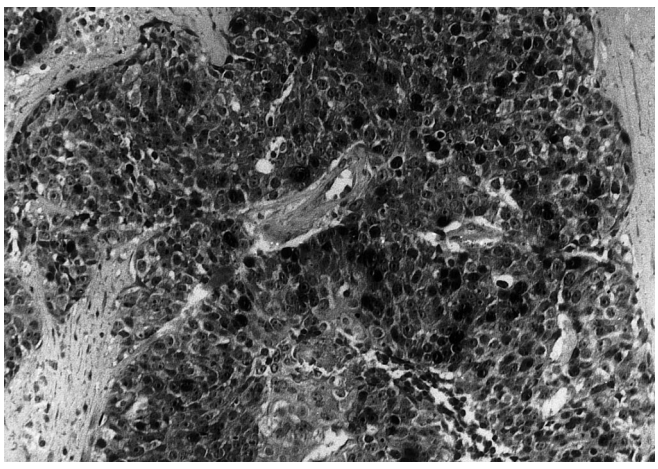

Fig. 1 Well-differentiated thymic carcinoma. Epithelial tumour cells with slight nuclear atypia, many of which are immunoreactive with the anti-p53 antibody CM-1: dark-stained nuclei show varying degrees of intensity. Original magnification x200

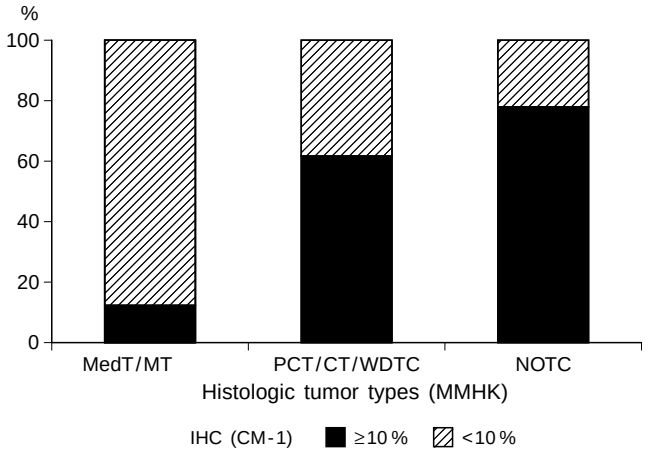

Fig. 4 Bar chart representing the distribution of immunohistochemical p53 detection in pathological tumour types. *MedT* medullary thymomas, *MT* mixed-type thymomas, *PCT* predominantly cortical thymomas, *CT* cortical thymomas, *WDTC* well-differentiated thymic carcinomas, *NOTC* non-organotypic thymic carcinomas. There is a significant difference between types in p53 detection: benign thymomas (medullary and mixed-type) showed a rare incidence of immunohistochemical p53 detection, whereas malignant thymomas (predominantly cortical, cortical thymomas, well-differentiated thymic carcinomas) and non-organotypic thymic carcinomas exhibited a high incidence of p53 detection ($P < 0.01$, Fisher's exact test)

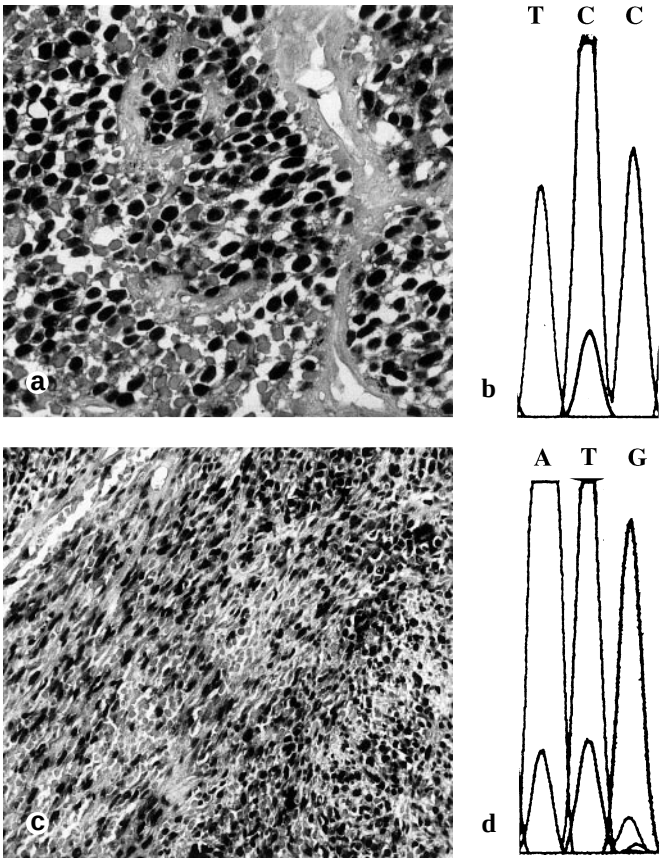

Fig. 5 **a** Non-organotypic thymic carcinoma, neuroendocrine differentiation. Most of the rounded epithelial tumour cells arranged in an organoid pattern reacted with the anti-p53-antibody CM-1: dark-stained nuclei. The same reaction pattern was obtained using the antibody D0-1 (data not shown). Original magnification x400. **b** DNA sequence analysis of exon 7 of the p53 gene in the neuroendocrine thymic carcinoma (sense strand), codon 242. There is a G→C transversion and loss of the wild-type allele: LOH. This transversion induces a cys→ser change during translation. **c** Non-organotypic thymic carcinoma, sarcomatoid differentiation. Most of the spindle-shaped epithelial tumour cells showed a CM-1-based immunoreaction: dark-stained nuclei. The same reaction pattern was obtained using the antibody D0-1 (data not shown). Original magnification x200. **d** DNA sequence analysis of exon 8 of the p53 gene in the sarcomatoid thymic carcinoma (antisense strand), codon 273. There is a C→T (G→A on the sense strand) transversion and loss of the wild-type allele: LOH. This transversion induces an arg→his change during translation

exact test. The effect of immunohistochemical results on survival times was determined by Kaplan-Meier survival curve estimates and log-rank test. Statistical analysis revealed a significant correlation ($P < 0.01$) between the above described incidence of CM-1-based anti-p53 immunoreaction and histological indicators of malignancy (Fig. 2). Further, CM-1 immunoreactivity correlated significantly ($P < 0.01$) with advanced clinical stages (Fig. 3). The impact of CM-1-based anti-p53-immunoreactivity on patients' survival was shown using Kaplan-Meier estimates (Fig. 4). There was a difference in survival between CM-1 immunoreactive and non-reactive cases: patients with immunoreactive thymomas showed substantially lower survival than those with non-reactive thymomas ($P < 0.05$).

Two cases were excluded from further investigation because DNA was not PCR amplifiable (1 non-immunoreactive well-differentiated thymic carcinoma and 1 non-immunoreactive mixed-type thymoma). Among the 42 amplifiable cases exon-specific PCR products were obtained and analysed by SSCP. Three tumours showed band shifts. Two band shifts were found in non-organotypic thymic carcinomas and one in a cortical thymoma. A neuroendocrine thymic carcinoma was characterized by a band shift in exon 7 of the p53 gene owing to a point mutation. DNA sequence analysis revealed a G → C transversion (Fig. 5b) at nucleotide 14052 (TGC→TCC) that is predicted to result in an aminoacid change at codon 242 (cytosine → serine). A sarcomatoid thymic carcinoma showed a band shift in exon 8. DNA sequencing revealed a G → A transition (Fig. 5d) at nucleotide 14487 (CGT→CAT) that is predicted to cause an aminoacid change at the hot spot codon 273 in the p53 protein (arginine → histidine) [12]. SSCP patterns in both cases were suggestive of loss of the homologous p53 allele (data not shown). Both cases were immunoreactive with the polyclonal antibody CM-1 and the monoclonal antibody D0-1. A cortical thymoma showed a band shift in exon 6 that was also present in whole-blood-derived DNA from the same patient. Both shifts were due to an A → G transition at nucleotide 13399 (CGA→CGG) affecting codon 213. The transition has no effect on translation and is consequently a silent

Table 3 Synopsis of results in immunohistochemistry and DNA analysis (*MMHK* histological classification of Marino, Müller-Hermelink und Kirchner [1985, 1992], *NOTC* non-organotypic thymic carcinomas, *WDTC* well-differentiated thymic carcinomas, *CT* cortical thymomas, *PCT* predominantly cortical thymomas, *MT* mixed-type thymomas, *MedT* medullary thymomas, *n* number of cases, *SSCP* single-strand conformation polymorphism, *T/N* tumour/normal tissue with same alteration, *LOH* loss of heterozygosity; numbers above nucleotides are serial numbers of nucleotides of coding and non-coding sequence of the p53 gene [39]

Histological subtype (MMHK)	*n*	Immune reactions p53-protein				DNA analysis p53 gene		
		Antibody D0–1		Antibody CM-1		SSCP band shift	Mutation	
		Reactive	Non-reactive	Reactive	Non-reactive		Codon	Nucleotide aminoacid
NOTC	9	4	5	7	2	2		
Squamous	1		1	1		–	–	–
Lymphoepithelial	2		2	2		–	–	–
Neuroendocrine	1	1		1		exon 7 +LOH	242	14052 G → C cys → ser
Sarcomatoid	1	1		1		exon 8 +LOH	273	14487 G → A arg → his
Not further classifiable	3	1	2	1	2	–	–	–
Undifferentiated	1	1		1		–	–	–
WDTC	7		7	5	2	–	–	–
CT	7		7	4	3	1 x exon 6 (T/N)	213 (T/N)	13399 A → G arg
PCT	4		4	2	2	–	–	–
MT	14		14	2	12	–	–	–
MedT	3		3		3	–	–	–

germline mutation of the p53-gene. No immunoreaction was observed in this case, either with CM-1 or with D0-1 antibodies (data not shown). Wild-type SSCP patterns for exons 5–8 were observed in all other immunoreactive and non-reactive cases. A perfect correlation was found between SSCP band shifts and point mutations (3/3). A synopsis of the results is given in Table 3.

Discussion

Thymomas present a challenge to the diagnostic pathologist, as some of them behave in a clinically malignant manner despite the absence of any cytological atypia. In past decades cytologically benign but invasive thymomas have prompted a bewildering variety of pathological classifications, which were mostly irrelevant to prognosis. When trying to predict the outcome of a thymoma the pathologist was limited to “useless guesswork” [21]. However, two competitive classification systems are prognostically relevant and are currently in use: the classification of Rosai and Levine and the MMHK classification (Müller-Hermelink et al. [8]). The revised classification of Rosai and Levine [2, 4] is mainly stage-oriented. Since the integrity of the thymic capsule may be disrupted by surgery or the invasion site might not be sampled this classification may be inadequate, especially in light of the finding that MMHK [8] is prognostically relevant [9-11, 22]. The MMHK classification circumvents the exclusive need for staging by applying cytohistological variables. Nonetheless, it remains a matter of controversy whether thymoma subtypes in this classification are biologically distinct entities and represent integral features in a tumour sequence.

In this study p53 appeared to differ in thymomas and non-organotypic thymic carcinomas. The immunohistochemical detection of p53 protein correlated with the main histological tumour types according to the MMHK system, Masaoka stages and patient survival times. Our data imply that p53 alterations are not involved in clinically benign thymomas (medullary and mixed-type thymomas). In the majority of malignant thymomas (predominantly cortical and cortical thymomas, and well-differentiated thymic carcinomas), altered p53 protein was identified by immunohistochemistry, but genetic alterations of the p53 gene were not detected. There are three possible explanations for these findings. First, in malignant thymomas, detectable amounts of p53 protein might reflect the augmented proliferation rate of these tumours, as p53 transcription is also activated during cell proliferation. Nevertheless, the immunohistological detection of p53 protein did not exhibit a random distribution but correlated with clinical stages and survival times. Secondly, different factors liable to interact with p53 could confer a stabilizing effect on the p53 protein such that its intranuclear quantity is raised to amounts detectable by immu-

nohistochemistry. Finally, p53 gene mutations might be located outside of the tested exons 5–8. However, mutations in p53 exons 1–4 and 9–11 and in introns seem to be rare events [13].

While both the monoclonal antibody D0-1 and the polyclonal antibody CM-1 recognized p53 equally well in colon carcinoma cells, there was a strikingly divergent reactivity pattern in thymomas. The polyclonal antibody CM-1 detected p53 in 62% of all thymomas and most non-organotypic thymic carcinomas, but in non-organotypic thymic carcinomas only p53 protein was detectable with the monoclonal antibody D0-1. This is the first report showing immunohistochemical divergent reactivity with CM-1 and D0-1 antibodies possibly due to the difference in p53 recognition by these antibodies. The polyclonal antibody CM-1 recognizes the entire p53 protein by binding to many different epitopes [16], whereas the monoclonal antibody D0-1 specifically binds to the N-terminal aminoacids 25–37 [23]. In CM-1-reactive and D0-1-non-reactive thymomas, this N-terminal region may be deleted by a truncation, but no such mutation events have been described so far [13]. Several viral proteins, such as SV40 large T antigen, HPV 16 and 18, E1B adenovirus, and the oncoprotein MDM2 are known to interact with p53. E1B and MDM2 bind to the N-terminal region of the p53 protein [24]. The MDM2-binding site maps to the N-terminal aminoacids 1–50, and the E1B-binding site is located between aminoacids 1 and 152. Both binding sites comprise the recognition site of the monoclonal antibody D0-1. Such MDM2–p53 and E1B–p53 complexes may hamper the immunoreaction with D0-1. MDM2 when overexpressed is known to be involved in a p53 autoregulatory feedback loop [25]. MDM2 overexpression has recently been detected in sarcomas that lack p53 mutations [26]. Lack of D0-1-based anti-p53 immunoreactivity and lack of p53 gene mutations in thymomas may therefore indicate a possible interaction between MDM2 and p53, suggesting that MDM2 may be a candidate to drive pathogenesis of thymomas.[1]

In contrast to thymomas, non-organotypic thymic carcinomas were partly reactive with both antibodies, and 50% of these cases also had p53 gene mutations. One missense point mutation found in a sarcomatoid thymic carcinoma hit codon 273 (exon 8), a hot spot site involved in many different human malignancies [13]. The other missense point mutation hit a neuroendocrine thymic carcinoma at codon 242 (exon 7). A third silent point mutation in codon 213 (exon 6) was identified as a non-tumour-specific but heterozygous germline mutation. Since the patient's cortical thymoma was not immunoreactive this finding was further evidence for the antibodies' specificity in detecting overexpressed and/or altered p53. Our results further imply that the immunoreaction with the polyclonal antibody CM-1 is not specifically related to p53 mutations in the exons 5–8, whereas the monoclonal antibody D0-1 seems to indicate mutated p53 proteins reliably.

[1] At the time this study was performed no reliable anti-MDM2 antibodies were available that would react with fixed tissues.

Our findings do not agree with those of a Japanese study that identified p53 overexpression and point mutations in thymomas of all clinical stages [27]. However, direct comparison is impeded by the usage of different antibodies and histological classification systems. As revealed by conflicting data on EBV involvement in thymic epithelial tumours [28, 29], these contradictory findings of p53 alterations in thymomas may reflect different tumour initiation events in different geographic areas.

By means of histology, clinical data, immunohistochemistry and DNA analysis we have identified three major entities of thymic epithelial tumours: benign thymomas with low detectable amounts of p53 protein and no p53 gene mutations; low-malignancy thymomas with detectable amounts of p53 protein and no p53 gene mutations and highly malignant non-organotypic thymic carcinomas with frequent occurrence of detectable amounts of p53 protein and p53 mutations at both homologous alleles.

CM-1-based immunohistochemical detection of p53 protein corroborates the MMHK classification of thymomas and points to a biological relationship between thymoma subtypes and a biological difference between thymomas and thymic carcinomas. CM-1-based immunohistochemical detection of p53 protein is considered useful in prediction of the growth behaviour of thymomas and their prognosis.

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Note added during the review process. Since this paper was submitted, Chen et al. have published a similar study on p53 immunoreactivity in thymomas [Chen FF, Yan JJ, Jin YT, Su IJ (1996) Detection of bcl-2 and p53 in thymoma: expression of bcl-2 as a reliable marker of tumour aggressiveness. Hum Pathol 27:1089–1092]. The immunoreaction in this study was performed with a monclonal anti-p53 antibody that binds to a different epitope of the p53 protein, and these findings do not contradict the results of the present study.