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Original Paper

KIT (CD117) is frequently overexpressed in thymic carcinomas but is absent in thymomas

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Abstract

KIT (CD117), a tyrosine kinase receptor, has not been widely studied in epithelial tumours. In a systematic immunohistochemical survey of KIT expression on tissue arrays incorporating 671 cases, it was found that thymic carcinomas frequently express KIT. Twenty-two thymic carcinomas, 110 thymomas, and 16 non-neoplastic thymus glands were retrieved for further analyses. Immunohistochemically, 19 (86%) thymic carcinomas revealed heterogeneous to diffuse membranous positivity, whereas no thymomas or normal thymus glands contained positive epithelial cells. Using reverse transcriptase-polymerase chain reaction (RT-PCR), *c-kit* transcripts could be demonstrated in all immunohistochemically positive cases. PCR amplification and direct sequencing of the *c-kit* juxtamembrane domains (exons 9 and 11) and tyrosine kinase domain (exons 13 and 17) were also performed on the thymic carcinomas but mutations were not found. Some non-thymic epithelial tumours showed frequent KIT expression including adenoid cystic carcinomas of the salivary gland (100% positive), chromophobe renal cell carcinomas (94%), renal oncocytomas (67%), and neuroendocrine tumours (34%). Other carcinomas were infrequently immunoreactive for KIT. The findings of this study suggest that KIT is involved in the pathogenesis of thymic carcinomas. The overexpression of KIT in thymic carcinomas has potential diagnostic utility in differentiating these tumours from thymomas and carcinomas arising from other sites, which express KIT infrequently.

Keywords: KIT; thymic carcinoma; thymoma; immunohistochemistry; mutation

Introduction

KIT (CD117) is a transmembrane tyrosine kinase receptor protein encoded by the proto-oncogene *c-kit* that maps to chromosome 4 (4q11–12) [1,2]. The KIT ligand is an early haematopoietic growth factor also known as kit ligand, steel factor, stem cell factor, and mast cell growth factor. After binding to the ligand, followed by homodimerization and phosphorylation, KIT begins an intrinsic signalling cascade that controls crucial mechanisms involved in cell proliferation, apoptosis, adhesion, and differentiation.

KIT is expressed at high levels in several normal tissues including haematopoietic stem cells, mast cells, melanocytes, germ cells, and the interstitial cells of Cajal [1]. Overexpression of KIT is also observed in a spectrum of human neoplasms, chiefly gastrointestinal stromal tumours (GISTs), myeloproliferative disorders, mast cell neoplasms, melanoma, and seminoma. In epithelial tumours, KIT expression has been reported in adenoid cystic carcinoma of the salivary glands [3,4], and occasionally in breast carcinomas [5,6], small cell (SCLCs) and non-small cell lung carcinomas [7–11], and gynaecological carcinomas [12,13].

In a systematic survey of KIT expression using a tissue array technique, we found that thymic carcinomas frequently overexpressed KIT, whereas thymomas were consistently negative. Thymoma is defined as an organotypic tumour with no or low-grade malignant features. Thymic carcinoma is a heterogeneous group of tumours with a malignant histological appearance [14]. The mechanisms by which thymic epithelial tumours develop are largely unknown. Distinction between thymic carcinoma and thymoma has clinical relevance, given their different prognoses and treatment protocols [15]. In this study, we confirmed our findings using immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR). We also attempted to detect mutations in the *c-kit* gene by PCR and direct sequencing.

Materials and methods

Thymic epithelial tumours and thymuses

During the period 1982–2002, 31 well-established cases of thymic carcinoma and 348 cases of thymoma were diagnosed at Taipei Veterans General Hospital. Nine of the 31 cases of thymic carcinoma had only

small biopsy samples, which made them unsuitable for this study. The remaining 22 thymic carcinomas, along with 110 randomly selected thymomas and 16 non-neoplastic thymus glands, were included in this study. The patients with thymic carcinoma consisted of 12 men and ten women, with ages ranging from 32 to 76 years (mean 58 years). The patients with thymoma consisted of 66 men and 44 women, with ages ranging from 23 to 78 years (mean 54 years). These cases had complete clinicopathological evaluation, including appropriate immunohistochemical stains and radiological studies, to rule out metastatic disease and to ascertain the thymic primary. Among the patients with thymic carcinoma, 15 patients died of disease (range of survival time: less than 1 month to 163 months; mean 47 months) and seven were alive (range of follow-up time: 37–217 months; mean 138 months). Among the 110 patients with thymoma, 22 patients died (range of survival time: 1–145 months; mean 52 months), 81 were alive (range of follow-up time: 1–226 months, mean 92 months), and seven were lost to follow-up.

The specimens were obtained through radical surgery, excisional biopsy or tumour debulking. The haematoxylin and eosin (H&E) slides from each case were reviewed and subtyped according to the 1999 WHO histological classification of thymic epithelial neoplasms [14]. The group of thymic carcinomas consisted of four keratinizing squamous cell carcinomas, seven non-keratinizing squamous cell carcinomas, nine lymphoepithelioma-like carcinomas, and two basaloid carcinomas. The thymomas comprised 26 type A, 18 type AB, 14 type B1, 37 type B2, and 15 type B3 tumours.

Tissue arrays

Five hundred and thirty-nine tumours of other types, predominantly epithelial tumours, were randomly selected from the same surgical pathology archive. The H&E-stained slides were examined under a light microscope. The areas of interest were circled with a colour pen. One to three foci per case were selected according to the heterogeneity of the tumour. We used a 16-gauge bone marrow biopsy trephine apparatus to punch the blocks at the circled areas. A tissue cylinder with 2.0 mm diameter was extracted. The cylinder was carefully transferred with forceps to a recipient metal paraffin block box. One recipient box could accommodate up to 88 (11×8) cylinders. After all the cylinders were aligned in the box, the box was covered with a plastic cassette and then liquid wax was gently poured into the box until it was full. The box was then put on a hot plate for 1 min to homogenize the wax, after which the box was removed from the hot plate and cooled to room temperature slowly. Four-micrometre sections were cut and mounted on silane-coated slides. A total of 1135 tissue cores were made into 13 blocks. The case types and numbers are tabulated in Table 1.

Table 1. Immunohistochemistry for KIT in a wide range of tumours

		Score			
Diagnosis	***n***	**0**	**1**	**2**	**3**
Thymic carcinoma	22	3	0	6	13
Thymoma	110	110	0	0	0
Renal cell carcinoma					
Conventional	26	26	0	0	0
Papillary	15	15	0	0	0
Chromophobe	16	1	3	7	5
Collecting duct	2	2	0	0	0
Unclassified	2	2	0	0	0
Renal oncocytoma	6	2	0	0	4
Nephroblastoma	7	5	2	0	0
Angiomyolipoma	23	23	0	0	0
Urothelial carcinoma	20	16	2	2	0
Salivary gland tumour*	40	25	0	3	12
Neuroendocrine carcinoma	44	29	5	6	4
Pulmonary squamous cell carcinoma	14	12	0	1	1
Pulmonary adenocarcinoma	12	11	0	1	0
Gastric adenocarcinoma	16	16	0	0	0
Colorectal adenocarcinoma	17	15	1	1	0
Hepatocellular carcinoma	28	28	0	0	0
Hepatoblastoma	4	4	0	0	0
Cholangiocarcinoma	7	7	0	0	0
Breast ductal carcinoma	21	17	1	2	1
Breast lobular carcinoma	12	12	0	0	0
Adrenocortical tumour	41	41	0	0	0
Phaeochromocytoma	21	21	0	0	0
Oesophageal squamous cell carcinoma	6	6	0	0	0
Pancreatic adenocarcinoma	11	11	0	0	0
Cervical squamous cell carcinoma	10	10	0	0	0
Endocervical adenocarcinoma	10	10	0	0	0
Ovarian surface epithelial tumour	30	30	0	0	0
Prostatic adenocarcinoma	25	25	0	0	0
Thyroid carcinoma	16	16	0	0	0
Mesothelioma	11	11	0	0	0
Germ cell tumour					
Seminoma	11	0	0	0	11
Yolk sac tumour	8	6	2	0	0
Choriocarcinoma	4	4	0	0	0
Embryonal carcinoma	3	3	0	0	0

* Positive cases were mostly adenoid cystic carcinomas.

Immunohistochemistry

The conventional slides of thymic epithelial tumours and the tissue array slides were stained for KIT (1 : 200; Dako, Carpinteria, CA, USA). The dewaxed slides were pretreated with 10 mM citric acid buffer, pH 6.0, and heated in a microwave oven for 10 min. The endogenous peroxidase was quenched by hydrogen peroxide and non-specific adsorption was reduced by swine serum. The bound antibodies were detected using the DAKO Envision system. This system is biotin-free. Immunopositivity was scored as 0, undetectable; 1+, heterogeneous positivity less than 50% tumour cells; 2+, strong positivity in 50–90% tumour cells; and 3+, diffuse positivity in more than 90% tumour cells. Control slides of selected cases of GIST were run in parallel.

Genomic DNA sequencing

The thymic carcinomas were evaluated for mutations in the juxtamembrane domains, exons 9 and 11, as well as in the tyrosine kinase domain, exons 13 and 17, by PCR and direct DNA sequencing. To ensure the greatest population of tumour cells, the tumour tissue was dissected under a light microscope and scraped down from dewaxed slides cut from the formalin-fixed, paraffin wax-embedded blocks. DNA was extracted following the standard procedure of proteinase K digestion, phenol–chloroform extraction, and ethanol precipitation. The intronic primers are listed in Table 2. PCR amplifications were performed using FastStart Taq DNA polymerase (Roche Molecular Biochemicals). The annealing temperature was 56 °C. The amplification products were electrophoresed on 2% agarose gels, purified from the gel, and sequenced using a fluorescent automatic sequencer (Prism 377 DNA sequencer, Applied Biosystems, USA).

RT-PCR of KIT transcripts

The tumour and adjacent normal tissue (residual thymus, soft tissue, lung or lymph nodes) of each case of thymic carcinoma were submitted for detection of *c-kit* mRNA by RT-PCR. RNAs were isolated using the Paraffin Block RNA Isolation Kit (Ambion, Huntingdon, UK) and cDNAs were synthesized using the SuperScript First-Strand Synthesis System (Invitrogen, Calrsbad, CA, USA) with random hexamers according to the manufacturers' protocols. The primers flank the joining region of exons 17 and 18 with an expected product size of 106 base pairs (Genbank: X06182). In genomic DNA, exons 17 and 18 are separated by an intron of 3247 base pairs (Genbank: U63834). The integrity of the cDNA was verified by amplification of the housekeeping β-actin gene (expected product size: 145 base pairs). The annealing temperature was 55 °C. The primers are listed in Table 2.

The materials used in this study consisted only of archival specimens from this department. We did not explore genomic sequences outside the scope of the study. The procedures followed the ethical standards of the human experimentation committee of the institute and the Helsinki Declaration of 1975, as revised in 1983.

Results

Immunohistochemistry

Nineteen thymic carcinomas (86%) revealed heterogeneous or diffuse membranous immunoreactivity for KIT (Figures 1A–1D). Thymomas of all histological subtypes (Figures 1E–1G) and non-neoplastic thymus glands (Figure 1H) were negative. Rare cases of thymoma revealed focal weak cytoplasmic immunoreactivity, which we interpreted as negative. Infiltrating mast cells served as an internal control.

Among the non-thymic tumours, seminomas, adenoid cystic carcinomas of the salivary gland, chromophobe renal cell carcinomas (Figures 1I and 1J), and renal oncocytomas (Figures 1K and 1L) were frequently positive. The positive rates were 100%, 100%, 94%, and 67%, respectively. About one-third (15/44) of neuroendocrine carcinomas, including eight carcinoids, one atypical carcinoid, three large cell neuroendocrine carcinomas (Figures 1M and 1N), and three small cell lung carcinomas (SCLCs), were positive. These positive cases originated from lung (nine cases), thymus (four cases), breast (one case), and uterine cervix (one case). Occasional immunoreactivity was observed in breast ductal carcinoma (4/21) (Figures 1O and 1P), nephroblastoma (2/7), urothelial carcinoma (4/20), pulmonary squamous cell carcinoma (2/14), pulmonary adenocarcinoma (1/12), colorectal adenocarcinoma (2/17), and yolk sac tumour (2/8).

PCR and RT-PCR

PCR amplification of the *c-kit* gene was successful for 21 thymic carcinomas. However, no *c-kit* mutation was found in any of the tumours that were analysed.

Satisfactory cDNA validated by the presence of β-actin transcripts was obtained in 20 cases. *c-kit* transcripts could not be amplified from one of these cases (case 3). This case was also non-reactive for

Table 2. Oligonucleotide primers used in this study

Primer	Sequence	PCR product size (bp)
Exon 9 forward	5′-TCC TAG AGT AAG CCA GGG CTT-3′	273
Exon 9 reverse	5′-GCC TAA ACA TCC CCT TAA ATT G-3′	
Exon 11 forward	5′-CCA GAG TGC TCT AAT GAC TG-3′	284
Exon 11 reverse	5′-AGC CCC TGT TTC ATA CTG AC-3′	
Exon 13 forward	5′-GCT TGA CAT CAG TTT GCC AG-3′	193
Exon 13 reverse	5′-AAA GGC AGC TTG GAC ACG GCT TTA-3′	
Exon 17 forward	5′-GTT TTC TTT TCT CCT CCA ACC TAA TA-3′	201
Exon 17 reverse	5′-TTG AAA CTA AAA ATC CTT TGC AGG AC-3′	
mRNA forward	5′-TAC TCA TGG TCG GAT CAC AAA-3′	106
mRNA reverse	5′-CCA CTT CAC AGG TAG TCG AGC-3′	
β-Actin forward	5′-GCT CGT CGT CGA CAA CGG CTC-3′	145
β-Actin reverse	5′-ACC AAC TGG GAC GAC ATG GAG AA-3′	

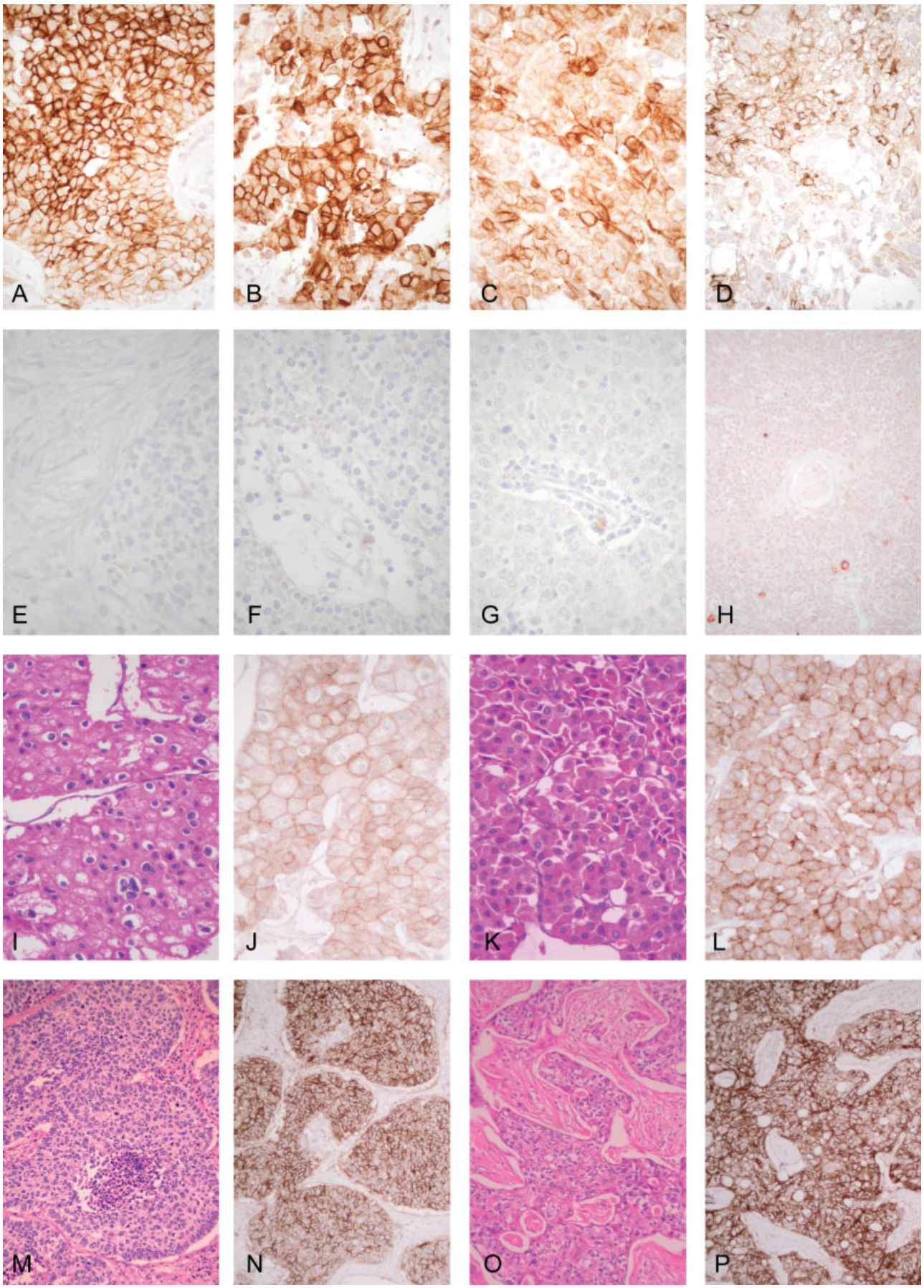

Figure 1. Immunohistochemical expression of KIT. Frequent overexpression was observed in thymic carcinomas (A–D), while thymomas (E–G) and normal thymus (H) were non-reactive. Overexpression was also observed in chromophobe renal cell carcinoma (I, J), renal oncocytoma (K, L), large cell neuroendocrine carcinoma (M, N), and ductal breast carcinoma (O, P)

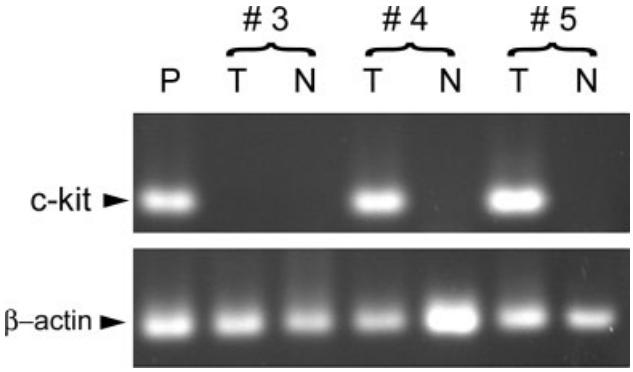

Figure 2. Examples of RT-PCR gel electrophoresis demonstrating *c-kit* transcripts in cases 4 and 5. Case 3 was negative. P = positive control; T = tumour tissue; N = non-neoplastic tissue

KIT protein immunohistochemically. *c-kit* transcripts were identified in tumour tissue from the remaining 19 cases, all of which were also positive for KIT immunohistochemically. No PCR product was identified from the corresponding non-neoplastic tissue (Figure 2).

Discussion

Compared with the plentiful articles concerning the association of KIT and GIST [1,16–23], studies addressing KIT expression in epithelial tumours are scanty. Apart from the reports addressing KIT expression in specific organs, a review of the relevant literature discloses two systemic immunohistochemical surveys of KIT on formalin-fixed, paraffin wax-embedded material from normal and neoplastic epithelial tissue. Tsuura *et al* [24] studied the expression of KIT in 1583 solid tumours, including 1489 epithelial tumours from different origins, and found that KIT was expressed in 36% of SCLCs but only sporadically in cervical carcinoma and non-SCLC. In contrast, Arber *et al* [12] studied 576 cases, including 387 epithelial tumours, and identified KIT expression in up to 53% of all cases examined. The overall prevalence of KIT expression in our series is close to that of Tsuura *et al*. The discrepancy might result from different sources of antibodies, dilutions, and the method of antigen retrieval, since all of these methodological variations can significantly affect KIT immunostaining [25,26]. It is notable that many positive cases in the series of Arber *et al* were described as having weak cytoplasmic immunoreactivity. The significance of this weak cytoplasmic positivity is uncertain. In the present study, we used control cases of GIST to optimize the dilution and antigen retrieval methods of KIT. With standardization of the staining parameters, we rarely observed such weak cytoplasmic staining.

In order to validate the immunohistochemical findings, we further performed RT-PCR to identify *c-kit* transcripts. Using paraffin wax specimens to detect RNA is more difficult than using frozen specimens; however, only archival paraffin wax specimens were available. We tried three different pairs of primers with expected product sizes ranging from 100 to 300 base pairs and the most satisfactory results were obtained when the amplicon was approximately 100 base pairs in length. The results of RT-PCR were fully compatible with those of immunohistochemistry. Although non-neoplastic tissue might contain a few infiltrating mast cells which express KIT, their number was too low to yield detectable *c-kit* transcripts in formalin-fixed and paraffin wax-embedded specimens.

We observed that KIT was preferentially expressed in a few subsets of epithelial tumour: thymic carcinomas, adenoid cystic carcinomas, chromophobe renal cell carcinomas, renal oncocytomas, and neuroendocrine carcinomas. Thymic carcinomas have a high frequency of KIT overexpression. On the contrary, thymomas do not express KIT immunohistochemically. This finding has not been noted before. Neither Tsuura *et al* [24] nor Arber *et al* [12] included thymus and thymic tumours in their systematic analyses. The overexpression of KIT in thymic carcinoma has diagnostic utility in discerning this tumour from other carcinomas, especially squamous cell carcinomas arising from the lung and oesophagus, which are the major differential diagnoses in this anatomical site. It is generally considered that thymic carcinomas encompass a heterogeneous group of carcinomas and that they lack distinctive histological features. Consequently, some authors claim that the diagnosis of thymic carcinoma can only be made by exclusion of other primaries [27]. However, there is evidence to indicate that thymic carcinomas may have a special immunohistochemical profile not shared by other morphological mimics. The immunoreactivity for CD5 [28] and CD70 [29] in thymic carcinomas, but not in non-thymic carcinomas, is such an example. Although KIT is not a specific marker for thymic carcinoma, the positive rate of KIT in thymic carcinomas was remarkably higher than that in pulmonary and oesophageal squamous cell carcinomas. In our series, the latter two tumours seldom expressed KIT immunohistochemically.

Thymomas are consistently negative for KIT, regardless of histological subtype. In this regard, we consider immunohistochemical staining for KIT to be a valuable adjunct for precise subtyping of thymic epithelial tumours, especially in the distinction between thymic carcinomas and type B3 thymomas. It is possible that true membranous immunoreactivity for KIT could be found in thymomas with more extensive sampling, but we believe that any such finding would be too focal to be of significance in contrast to the more obvious presence in thymic carcinoma. Accordingly, we recommend that immunostaining for KIT should be included in the immunohistochemical panel for the diagnostic assessment of mediastinal epithelial tumours. Since no single currently available marker is absolutely discriminatory, it is judicious to employ more than one immunostain. Other helpful markers include those that are frequently positive in thymic carcinoma such as CD5 [28] and Bcl-2

[30]. Both thymic carcinoma and thymoma consistently express p63 [31,32]. CD1a and CD99 immunostains aid in labelling the immature thymocytes that are usually present in thymoma but not in thymic carcinoma and non-thymic neoplasms [33]. Because thymic carcinoma is morphologically non-organotypic, it is of utmost importance to exclude the possibility of metastatic disease. Relevant markers suggestive of possible origins are contributory; for example, thyroid transcription factor-1 to rule out pulmonary carcinomas [33] and keratin profile (eg keratins 5/6, 7, and 20) to provide a guideline for possible origin [34].

A caveat about this study is that the current methodology cannot determine the functional status of KIT protein. The underlying mechanism for the overexpression of KIT in thymic carcinomas is unknown. KIT cannot be regarded as a native marker for thymic epithelial cells, given the absence of KIT-positive epithelial cells in non-neoplastic thymus. The common KIT genomic mutations that lead to its constitutive activation in GISTs [16,17,20,21,23], myeloproliferative disorders [35,36] or mast cell diseases [37,38] are not present in thymic carcinomas. Elucidation of this point has clinical implications for evaluation of the eligibility of patients for a therapeutic trial of the tyrosine kinase inhibitor STI-571 [39]. Further phosphorylation studies [20] are necessary.

We found that chromophobe renal cell carcinomas and renal oncocytomas frequently express KIT. Our finding is in agreement with the recent study of Yamazaki *et al*, who reported, using high-density oligonucleotide arrays targeting 12 000 genes, that the *c-kit* oncogene was up-regulated specifically in chromophobe renal cell carcinomas [40]. The potential usefulness of this marker for the classification of renal tumours requires studies of larger series. We also noted that a proportion of neuroendocrine carcinomas of different origins expressed KIT. The positivity was not limited to SCLC, but also covered carcinoid and large cell neuroendocrine carcinoma [7]. Whether the positive tumours differ biologically from their negative counterparts is intriguing. The genetic and biological aspects of KIT expression and its subsequent clinicopathological significance in epithelial tumours are a field worth exploring.

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