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Thymic epithelial tumour progression in an SV40T transgenic mouse model

Cortical thymoma–thymic carcinoma sequence

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Abstract There have been several reports that thymoma in human is a progressive disease, and that thymoma and thymic carcinoma form a continuum. We established a stable line of SV40T transgenic mice, which consistently produced thymic epithelial tumours progressing to thymic carcinoma within a predictable time span. Using this animal model and a morphological approach, thymic epithelial tumour progression was studied with reference to sequential changes at different time points in animals aged from 3 to 32 weeks. At all ages, SV40T was expressed in the nuclei of thymic epithelial cells; in these transgenic mice we observed the entire spectrum from cortical type thymoma to thymic carcinoma. Thymic size tended to increase with ageing in SV40T TG mice. While younger mice had predominantly cortical (organoid) or cortical thymoma, older mice had well-differentiated thymic carcinoma (WDTC) or poorly differentiated thymic carcinoma. When SV40T TG mice (248 line) reached a certain age, carcinoma of the thymus was present in all of them. Cortical-type thymoma became malignant within a predictable time span, suggesting a cortical thymoma–carcinoma sequence. When the mice were 9 weeks of age, the thymuses formed gross masses compatible with cortical thymoma. At 14 weeks of age, WDTC appeared against the background of cortical thymoma. Poorly differentiated thymic carcinoma was found after 15 weeks and affected all animals over 23 weeks of age. Most thymic carcinomas coexisted in varying proportions with cortical-type thymoma. Medullary thymomas did not develop in the mice, and no transition from medullary-type thymomas to thymic carcinomas was observed. In this SV40T transgenic mouse model, thymic carcinoma is clearly preceded by cortical-type thymoma. These transgenic mice may provide an interesting model for the progression from cortical thymoma to WDTC and/or high-grade carcinoma.

Key words SV40T · Thymoma · Thymic carcinoma · Thymus · Transgenic mouse

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Introduction

The large T antigen of simian virus 40 (SV40T) is a nuclear protein necessary for the synthesis of DNA and is a potent oncogene, determining both immortalization and transformation of multiple cell types in culture systems and in vivo [8, 25]. Transgenic mice (TG mice) offer the potential for studying the biological effects of gene expression under physiological conditions that cannot be reproduced in culture, thus providing a system that may give an accurate simulation in differentiated tissues of such pathologic processes as neoplastic transformation [9]. A TG mouse harbouring the SV40T gene was used as an animal model of spontaneous carcinogenesis by regulating its expression with a variety of transcriptional elements. Choroid plexus tumours and pancreatic islet cell tumours have developed in most studies involving SV40T TG mice [5, 7, 10, 12, 36, 49, 56]. In addition, SV40T antigen induced a variety of neoplasm including – for example – lymphoma, rhabdomyosarcoma and osteosarcoma [1, 4, 6, 11, 20, 28, 32, 46, 47, 53, 61], depending on the transcriptional signals used for their expression. Thymic hyperplasia was observed incidentally in some TG mice that produced choroid plexus tumours [5, 7, 36, 49] and also as a main event in TG mice har-

Table 1 Summary of thymic mass shown in SV40T transgenic mice (*UD* undetermined nature, *CT*: cortical type (cortical or predominantly cortical) thymoma, *WDTC*: well-differentiated thymic carcinoma against the background of cortical-type thymoma, *TCa*: thymic carcinoma with or without WDTC and/or thymoma)

Mouse group		Age (weeks)	Number of cases	Mass size (cm)			Histological diagnosis			
				<1	1–1.5	>1.5	A (UD)	B (CT)	C (WDTC)	D (TCa)
Nontransgenic		4–30	7	7						
Transgenic	I	3–5	5	5	0	0	4	1		
	II	6–7	4	4	0	0	1	3		
	III	9–10	5	1	2	2		5		
	IV	12–14	10	0	4	6		8	2	
	V	15–16	10	0	3	7		4	3	3
	VI	17–19	8	0	1	7		1	4	3
	VII	20–21	20	0	4	16		3	1	16
	VIII	23–32	8	0	0	8				8

bouring a growth hormone-releasing factor (GRF) promoter fused with SV40T [3, 34]. Teitz et al. [57] recently reported a mixed-type thymoma in TG mice expressing SV40T under the control of an erythroid-specific enhancer. There has been a report that not only those expressing SV40T, but also Thy1-myc TG mice developed complex lymphoid and epithelial thymic tumours [50]. We established a stable line of TG mice which, in all cases, consistently produce thymic epithelial tumour progressing to thymic carcinoma within a predictable time span [38].

Thymic hyperplasia refers to an increase in thymic mass that leaves the basic framework and histological appearance intact [22, 44]. Thymoma is defined as an epithelial tumour of the thymus with a variable number of reactive lymphocytes [22]. Morphological diversity, together with poor correlation of histology and clinical behaviour in the past, has led to numerous classifications for thymomas. They are traditionally classified with reference to the ratio between epithelial cells and lymphocytes [22, 45], but this system has no advantage over clinicopathological correlation and fails to point out that only epithelial cells are neoplastic. Levine and Rosai [26] proposed a simplified classification: benign thymoma, malignant thymoma category I and malignant thymoma category II. It is limited, however, in that there is no histological difference between benign thymoma and malignant thymoma I. An alternative classification based on morphological features and histogenesis of the epithelial components was proposed by Marino and Müller-Hermelink [29], and revised by Kirchner and Müller-Hermelink [18]; it included medullary, mixed, predominantly cortical, cortical, and well-differentiated thymic carcinoma. In all classifications, thymoma and thymic carcinoma have been classified as distinct entities on the basis of morphology. Thymoma has many organotypical features, such as perivascular spaces, epithelial palisading, medullary differentiation, and Hassall's corpuscles [23]. Thymic carcinoma, however, bears no resemblance to normal thymic epithelium, with its obvious cytological atypia and necrosis [55, 58]. In a number of thymic epithelial tumours there is a degree of cytological atypia intermediate between conventional thymoma and thymic carcinoma, together with signs of organotypical differentiation. Such tumours have recently been defined as well-differentiated thymic carcinoma (WDTC), which is regarded as an organotypical low grade carcinoma [18, 19, 24, 37, 39, 42].

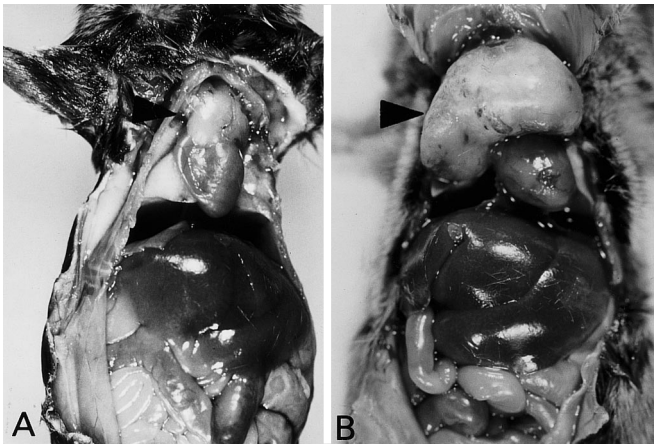


Fig. 1A, B Gross photographs in situ. **A** Enlarged thymus (*arrowhead*) of a 6-week-old transgenic (TG) mouse harbouring SV40T. Thymuses enlarged to about 5 times that of age-matched control mice. **B** The thymic mass (*arrowhead*) at 20 weeks of age. A large ovoid mediastinal mass is pushing the heart and lungs backwards

There have been some human case reports of thymic carcinoma associated with thymoma [13, 14, 23, 35, 48, 52, 55, 60]. Suster and Moran [54] recently reported 22 cases of thymic epithelial neoplasm characterized by an admixture of thymoma and thymic carcinoma. They suggested the existence of a continuum of differentiation from thymoma to thymic carcinoma.

In laboratory animals, spontaneously occurring thymomas are uncommon [22, 51]; there is no animal model in which thymoma or the thymoma–carcinoma sequence might be studied. Ideally, the TG mouse model of thymic epithelial tumour should exhibit a heritable and reproducible pattern of lesion development, perhaps with separate steps in a sequence of events culminating

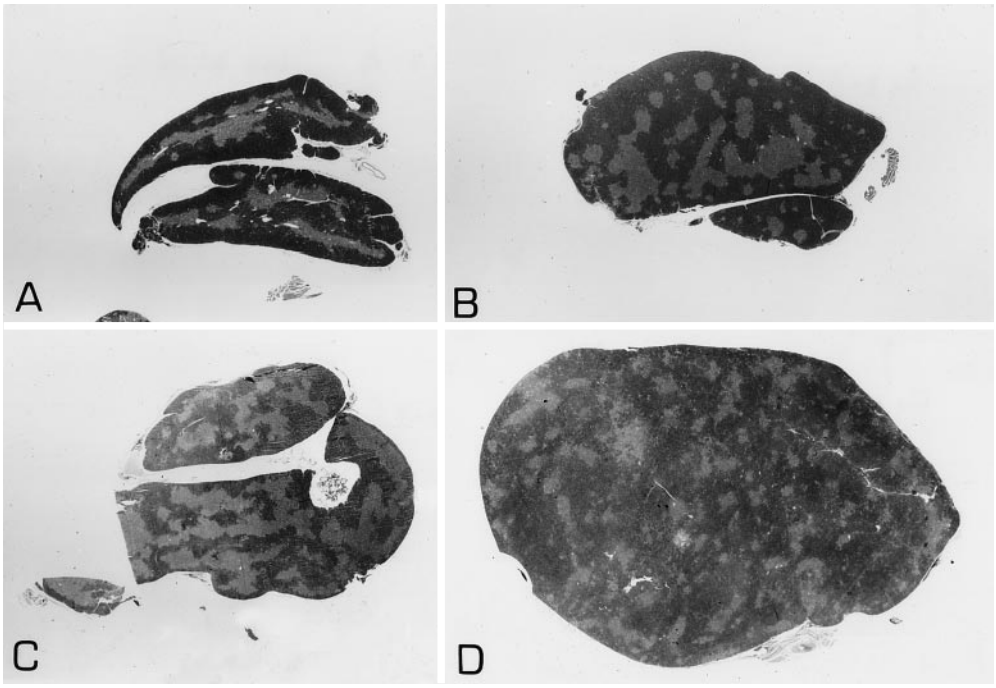


Fig. 2A–D Low-power view of thymus of TG mice and non-transgenic control mice in different periods. H&E, ×2.5. **A** Thymus of non-TG control mouse shows normal thymic architecture with preserved outer-cortical/inner-medullary pattern. **B** Period I TG mice (3–5 weeks): slightly enlarged thymus shows an irregular arrangement of cortical and medullary epithelial zones, with clear distinction between the two. The cortical lymphocyte zone is relatively expanded. **C** Period II (6–7 weeks): irregular, further expanded, medullary zone. **D** Period III (9–10 weeks): predominantly cortical thymoma

in neoplasia. We attempted to elucidate the pathogenesis of thymic carcinoma using such an animal model, with 100% penetration by thymic carcinoma. We studied sequential thymic change in SV40T TG mice by means of a morphological and immunohistochemical approach at different time points in animals aged between 3 and 32 weeks and tried to provide evidence for the transition from benign epithelial thymoma to thymic carcinoma, identifying thymic epithelial cell changes during malignant transformation and their time sequence.

Materials and methods

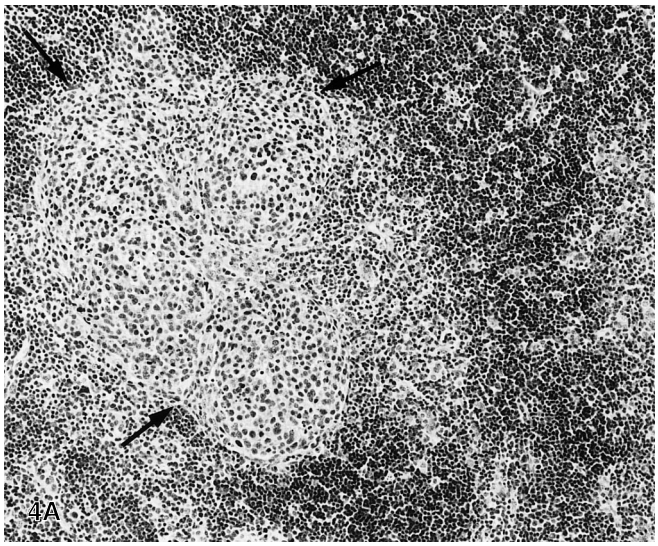
A 3.0-kb BamHI-KpnI fragment carrying the SV40 early region gene was cloned into the multicloning site of pUC19 DNA vector (pSVT). This plasmid contains a SV40 large T and small t antigen under the control of its own enhancer-promoter region. For microinjection into the fertilized egg, pSVT DNA was linearized with SalI digestion and 5.7-kb DNA fragments including vector sequence were isolated using a glass bead (Qiagen, USA).

The SV40T transgene in pSVT DNA was micro- injected into the male pronucleus of the fertilized zygotes obtained from F1 hybrid mice (C57BL × CBA). Eggs were then implanted into a pseudopregnant ICR foster mother as previously described [16]. The offspring were screened for the presence of the transgene by Southern blot analysis or PCR for SV40 T gene in DNA isolated from the tails as described elsewhere [5]. Transgene positivity was maintained by crossing the mice in which this was found with normal C57BL/6J mice or positive littermates.

To study the progressive development of lesions in the thymus of the 248 TG line, mice were sacrificed between 3 and 32 weeks after birth (Table 1), after confirmation of the integration of SV40T to mouse DNA. A total of 70 TG mice were examined and 7 transgene-negative littermates of mice from the 248 line were analysed in parallel as controls for the different stages of tumourigenesis. All experiments were conducted according to National Institute of Health guidelines for animal care and experimentation.

After the whole thymus had been removed, samples were fixed in 10% buffered formalin for light microscopy and a proportion were snap-frozen in cooled isopentene in liquid nitrogen, and kept in liquid nitrogen until used for immunohistochemistry. Samples of other solid organs such as the liver, spleen, lung, kidney, testis, salivary gland and brain were also examined light microscopically. The frozen tissue was embedded on OCT compound for sectioning; 6 μm sections were fixed for 5 min in ice-cold acetone.

TG mice were divided into eight age-dependent periods (period I–VIII). Each thymus was measured and weighed, and paraffin sections were stained with H&E. Three pathologists examined the thymus lesions independently, and the thymoma classification based on the Müller-Hermelink system [18, 19, 29, 40] was adopted. Thymic epithelial tumours were categorized as medullary thymoma, mixed thymoma, predominantly cortical (organoid) thymoma, cortical thymoma, WDTC, or conventional thymic carcinoma. Cortical thymomas were composed of medium and large epithelial cells with round to oval nuclei; nuclear chromatin was loose and stippled, and one centrally located nucleolus was usually present. Organoid structures were often observed, and the population of lymphocytes was quite variable. The epithelial cells of medullary thymomas were medium sized and contained an oval- to spindle-shaped nucleus; the chromatin was fine and evenly distributed, and the nucleolus was always inconspicuous. The lymphocytes were generally fewer in number than those of cortical thymoma. Mixed thymomas were made up of a mixture of both elements. Organoid thymoma, as proposed by Pescarmona et al. [40], was a distinct variant of cortical thymoma. It mimicked the architecture of normal thymus and was characterized by a ‘starry-sky’ appearance and the presence of diffuse areas of medullary differentiation with or without Hassall’s corpuscles. The diagnosis of WDTC was based on morphological criteria such as a clear predominance of epithelial cells, which exhibit a solid growth pattern and contain organotypical features such as epithelial palisading or perivascular spaces. The round to oval epithelial cells of WDTC have mild to moderate cytological atypia and occasional mitotic figures, and the tumour contains fewer lymphocytes than cortical thymoma [18, 19, 37, 41, 43]. Thymic carcinoma was defined according to the morphological criteria of Troung et al. [58]: frequent mitoses (more than 10/10 high-power fields), tumour cell necrosis, a virtual absence of lymphoepithelial mixture, and nuclear atypia including large cell size, high nuclear/cytoplasmic ratio and prominent nucleoli.



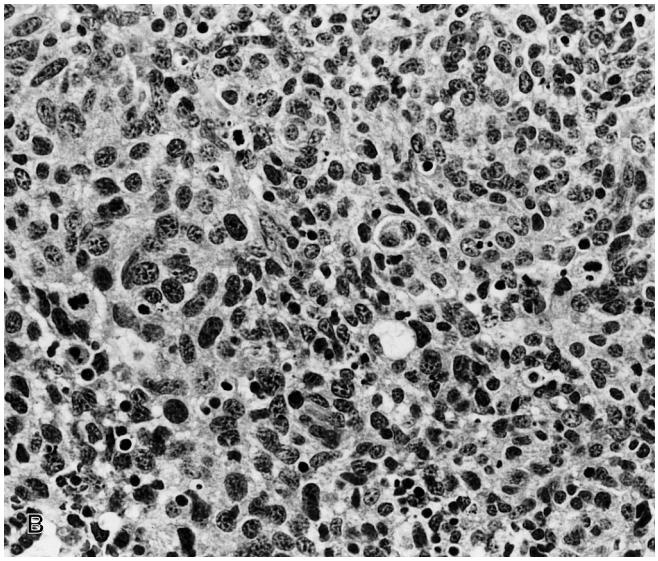


Fig. 3 Organoid thymoma showing starry-sky appearance and medullary differentiation at 12 weeks. H&E, ×13

Fig. 4 **A** Atypical epithelial cell focus (*arrows*) is noted against the background of a thymoma. H&E, ×33. **B** High magnification of the malignant focus. It is made up of tumour cells with a large ovoid vesicular nucleus and frequent mitoses. H&E, ×100

Thymic lesions of TG mice were divided into four groups on the basis of histological features, as follows: group A with enlarged thymus of undetermined nature; group B with cortical type thymoma (cortical or organoid); group C with the presence of WDTC against a background of cortical-type thymoma, and group D with thymic carcinoma with areas of WDTC and/or cortical-type thymoma.

To localize the antigen, polyclonal rabbit anti-SV40T antibody (161-T) provided by Dr. Loren J. Field (Indiana University, Indiana, USA) was used [21]. Anti-SV40T antibody (161-T) was applied overnight at 4°C to both frozen and paraffin-embedded thymic tissue using the ABC method. SV40T was demonstrated by nuclear staining.

To confirm the epithelial nature of the component cells, polyclonal anti-cytokeratin (Zymed) was added to paraffin sections of all cases. To determine whether epithelial cells were cortical or medullary, Th-3, monoclonal antibody for stromal cells of thymic cortex, Th-4, monoclonal antibody for stromal cells of thymic medulla, and rat monoclonal antibody for anti-mouse thymic epithelial cells (Biosource, MS-017-SN) were applied to the frozen tissue section of thymic lesions. The monoclonal antibodies, Th-3 and Th-4, were provided by Dr. K. Hirokawa (Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan) [15]. The routine ABC method was used, with primary antibody application for 2 h at room temperature, and positive staining for cytoplasmic membrane was seen.

Results

Thymic changes in SV40T TG mice are summarized in Table 1. Before 5 weeks of age (period I), the thymus did not form tumour-like masses, but it became slightly bigger and heavier than the thymuses of transgene-negative littermates. On gross examination, diffuse enlargement with preserved lobular architecture was seen. By 6–7 weeks (period II), the size and weight of thymuses were twice what they had been in period I (Fig. 1A) and by 9 weeks of age (period III) they had enlarged markedly and were up to 25 times the size of the control thymus. They measured more than 1 cm in maximal diameter and formed gross tumour masses without lobular architecture. As shown in Table 1, the thymus enlarged progressively with age, and by 20 weeks its size was up to 2 cm (Fig. 1B); larger thymic masses often showed grossly visible necrosis. In three animals, lung metastases were detected.

In general, microscopic changes correlated well with gross findings. In all seven age-matched control mice the thymic architecture was normal; the cortico-medullary architecture was preserved (Fig. 2A).

Period I (3–5 weeks). Four mice with a slightly enlarged thymus showed irregularity of the arrangement in the cortex and medulla (Fig. 2B); the corticomedullary junction was clear and distinct, however. In these cases, the cortical lymphocyte zone had expanded more than in their normal littermates. Large lymphoid cells (lymphoblasts) were the main element of the expansion, and these, mixed with small thymocytes, showed occasional mitoses. Epithelial elements in the cortex and medulla remained in normal proportion in these four cases. One case, however, showed an increased number of epithelial cells in the medulla.

Period II (6–7 weeks). The corticomedullary architecture was disturbed and showed an irregular arrangement, as in period I. Thymuses in this group showed an irregu-

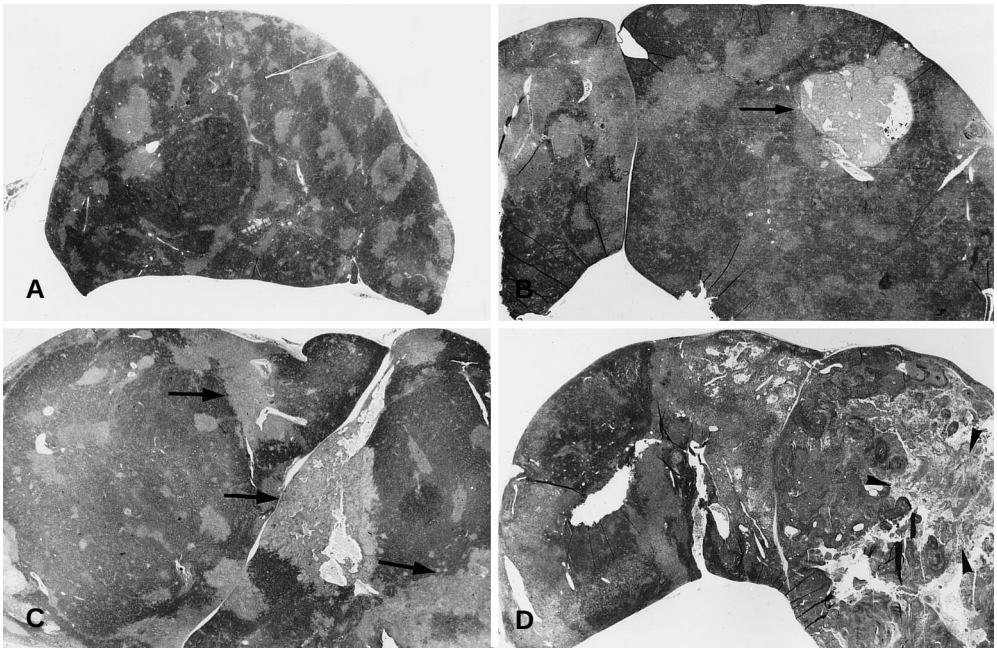


Fig. 5A–D Low-power view of thymus after 12 weeks of age. H&E, ×2.5. **A** Epithelial zone expanded markedly in period IV (12–14 weeks). **B** Periods V and VII (after 15 weeks): malignant epithelial focus (*arrow*) in the background of cortical thymoma. **C** Well-differentiated thymic carcinoma (WDTC) against the background of cortical thymoma at 18 weeks. **D** Mixed pattern of thymic carcinoma (*right*), WDTC (*centre*, and cortical thymoma (*left*) at 21 weeks

larly expanded medulla, particularly compared with those of animals in period I (Fig. 2C). The number of epithelial cells had increased considerably. It was difficult to classify them as thymoma or thymic hyperplasia. Therefore they were categorized as *undetermined*; however, by definition they were consistent with thymoma rather than thymic hyperplasia.

Period III (9–10 weeks). All five period III cases were compatible with the predominantly cortical thymoma of the Müller-Hermelink system [18, 40]. They showed medullary differentiation manifested by pale foci reminiscent of medullary areas of normal thymus within a neoplastic tissue resembling the cortex (Fig. 2D). In some cases there was a starry-sky appearance, and Hassall's corpuscles were occasionally found (Fig. 3). Epithelial cells with a vesicular nucleus had increased in number individually or were in small clusters of cells.

Period IV (12–14 weeks). The most prominent finding in this period was the emergence of malignant epithelial foci against the background of cortical or predominantly cortical thymoma. This occurred in two of the ten mice examined. The malignant foci were made up of tumour cells with large ovoid vesicular nuclei resembling those of thymic cortical cells, and with some areas exhibiting an organoid pattern. They were therefore compatible with WDTC, despite the presence of more than ten mitotic figures per high-power field (Fig. 4). In the other eight animals and elsewhere in the thymuses of the two mentioned above, epithelial zones were markedly expanded (Fig. 5A) and consisted of cortical-type epithelial cells and thymocytes. Mitoses were occasionally detected in the expanded epithelial zone. Scattered large cortical-type epithelial cells were found not only in this zone but also throughout the lymphocyte-predominant area, and these were compatible in appearance with human cortical thymoma (Fig. 6).

Period V (15–16 weeks). In this period, three of ten mice showed poorly differentiated thymic carcinomas with extensive necrosis, and coexistence with cortical thymoma and/or WDTC. Three others showed WDTCs (Figs. 7–9), and they combined WDTC and background cortical thymoma, with the extent in each case varying from multiple small foci to 30% of the mass (Fig. 5B, C). The four thymuses with no carcinoma were consistent with cortical or predominantly cortical thymoma.

Period VI (17–19 weeks). One case was predominantly cortical thymoma, four were WDTCs and the other three were mixed WDTC and poorly differentiated thymic carcinoma against the background of cortical thymoma.

Period VII (20–21 weeks). Six of twenty mice were suffering from poorly differentiated thymic carcinoma, three mainly from WDTC, and seven showed a mixed pattern of WDTC and poorly differentiated thymic carcinoma (Fig. 5D). In all these sixteen mice, association with cortical-type thymoma was seen. One case showed a focus of WDTC; the remaining three cases had cortical thymoma only.

Period VIII (23–32 weeks). All eight thymuses examined in this period were poorly differentiated thymic carcinomas combined with cortical thymoma and/or WDTC. In seven cases, thymic carcinomas coexisted with WDTCs.

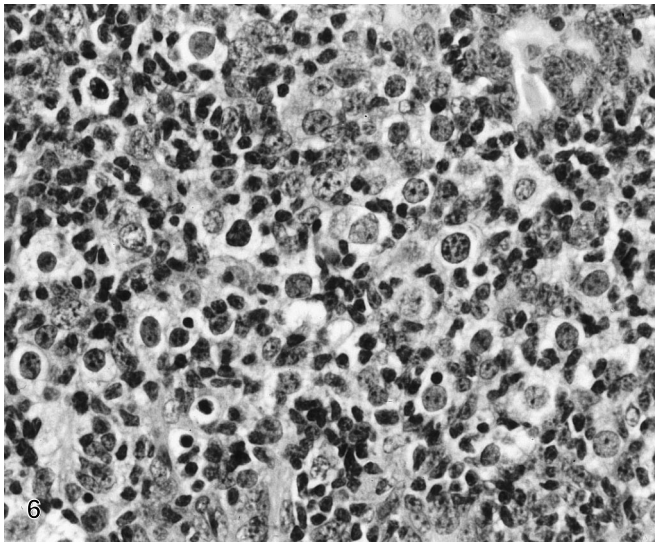
Thymic carcinomas in TG mice all had basically the same histology, showing poor differentiation with regard to any conventional classification of thymic carcinoma. It was difficult to classify them into squamous cell carcinomas, sarcomatoid carcinomas, undifferentiated carcinomas, or any other category, and we therefore named them "poorly differentiated carcinomas". Most thymic carcinomas showed combined features and transitional zones of ovoid to spindle-shaped cells and large ovoid cells mimicking thymic cortical epithelial cells (Fig. 10).

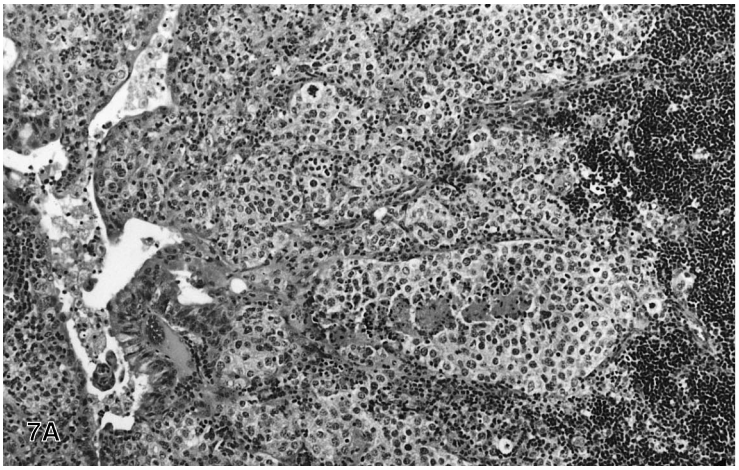
Fig. 6 In cortical thymoma, an expanded epithelial zone showed an increase in cortical-type epithelial cells with round vesicular nuclei, mixed with lymphocytes. H&E, ×100

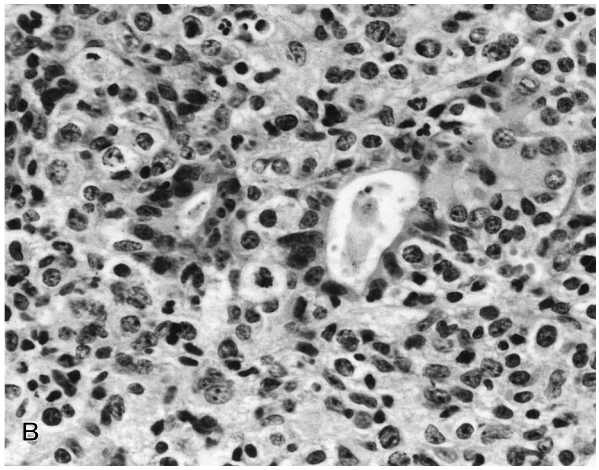
Fig. 7A, B Gland-like palisading pattern in the solid sheet of ovoid epithelial cells in WDTC. H&E, **A** ×25, **B** ×100

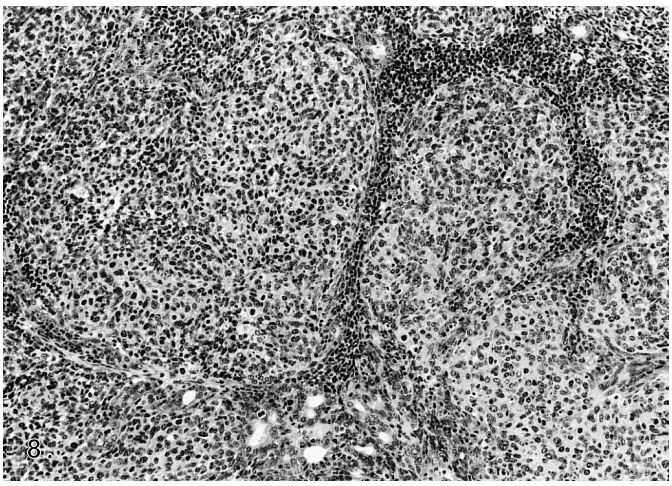
Fig. 8 Lobulating pattern surrounded by lymphocytes in WDTC. H&E, ×40

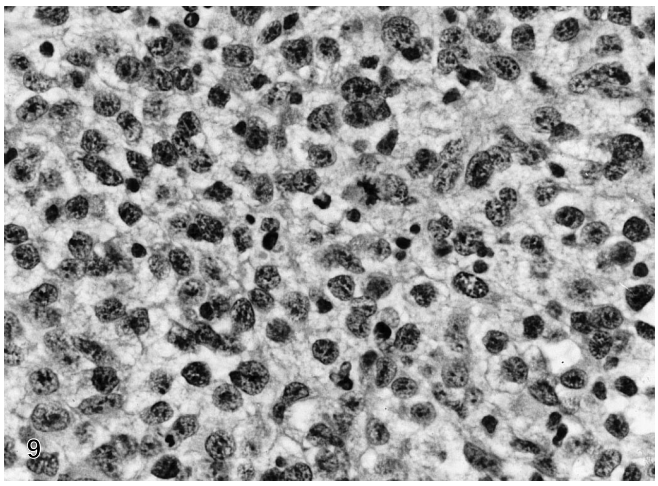
Fig. 9 Cells with large ovoid vesicular nucleus in WDTC. H&E, ×100











In contrast with WDTCs, poorly differentiated thymic carcinomas were characterized by obvious necrosis and numerous mitoses. A few cases showed focal squamous differentiation and could be classified as poorly differentiated squamous cell carcinoma. In the areas of large ovoid carcinoma cells thymic carcinoma appeared, merging into cortical thymoma or WDTC.

Expression of the SV40T transgene in tissues was determined by Northern blot analysis and immunohistochemistry. A relatively high level of expression of the 5.7-kb transcript was detected in the thymic mass and kidney of mice in all groups (Fig. 11). The expression level was low in the spleen. Northern blot analysis showed that the level of SV40T expression in thymic

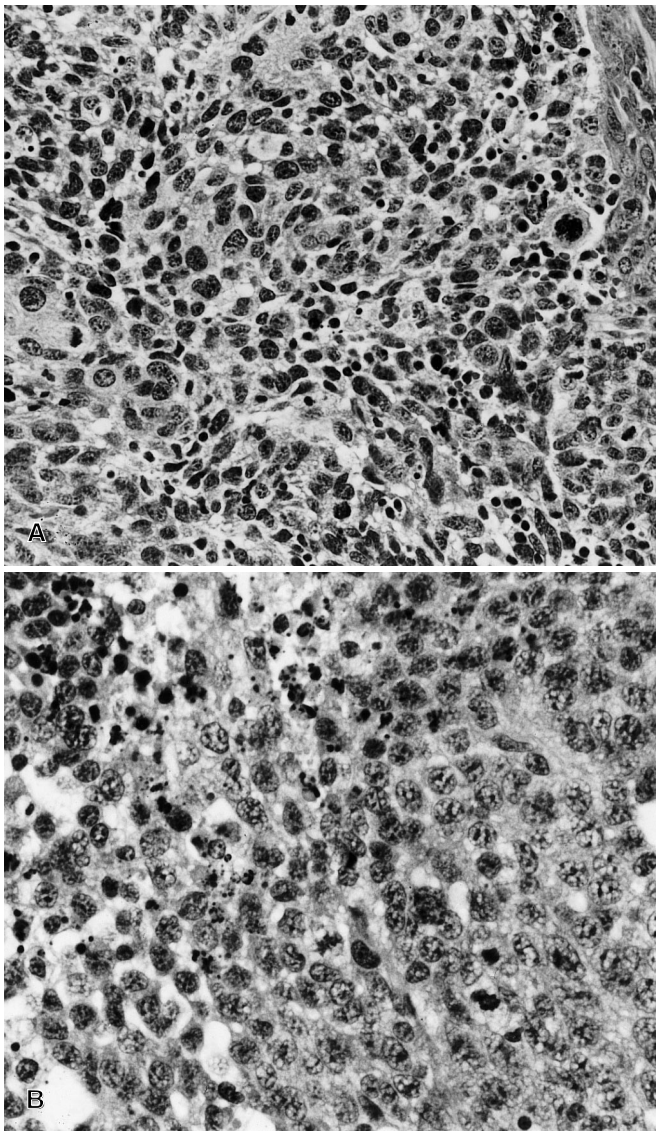


Fig. 10 Poorly differentiated thymic carcinoma. H&E, **A** ×80, **B** ×100

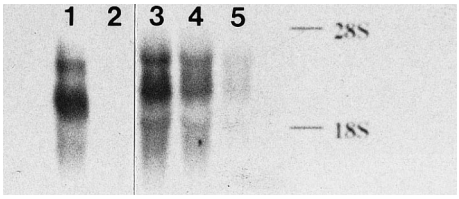


Fig. 11 Northern blot analysis for SV40T in TG mice, of total RNAs from RHEK (*lane 1* keratinocyte cell line transformed by SV40 T), liver (*lane 2*), thymus (*lane 3*), kidney (*lane 4*) and spleen (*lane 5*) of transgenic mice. Thymus (*lane 3*) and kidney (*lane 4*) of TG mice showed positive bands, with the same sites as *lane 1* (positive control)

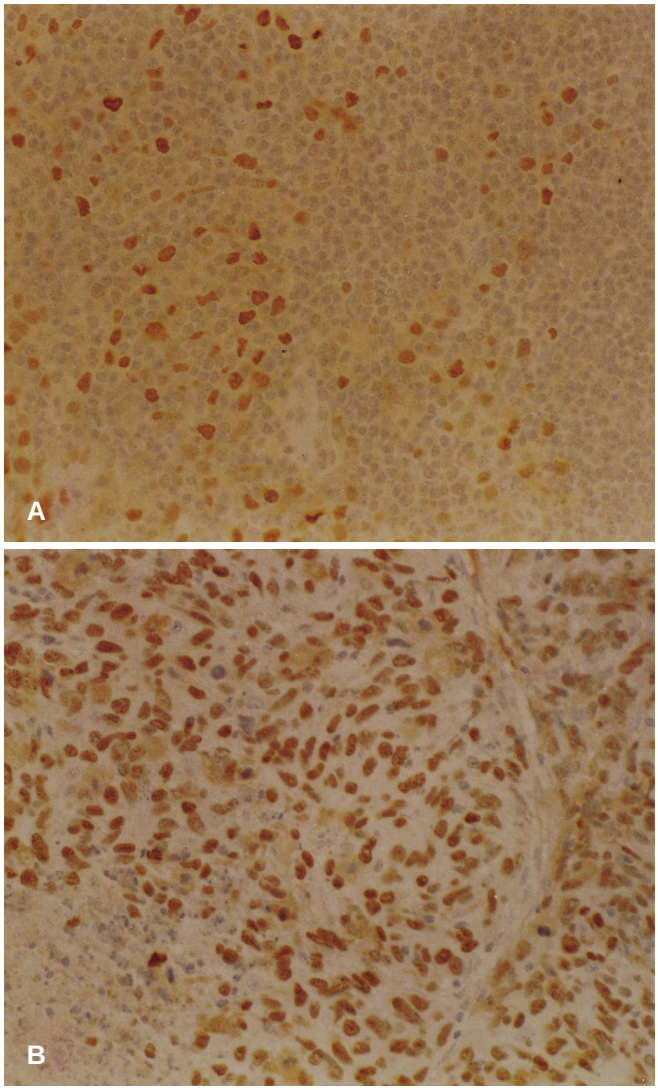


Fig. 12 Immunohistochemical staining for SV40T in **A** cortical thymoma and **B** thymic carcinoma. SV40T showed nuclear staining on most epithelial cells

carcinoma was higher than in thymoma, and this corresponded with the immunohistochemical results. On immunohistochemistry, SV40T showed strong nuclear expression in thymic epithelial cells on frozen and paraffin sections through all experimental periods (Fig. 12). Thymocytes seemed not to express SV40T. In addition, dysplastic tubules and adenomatous kidney lesions also strongly expressed SV40T. Negative control thymuses of non-TG mice did not show SV40T expression.

In thymoma, cytokeratin was positive for scattered clusters of epithelial cells. As the age of the mice increased, the cytokeratin-positive cells gradually increased until carcinoma was clearly evident. In carcinomas, cytokeratin stained in some tumour cells in small foci, but the majority of carcinoma cells revealed no staining. Gland-like structures in WDTC were invariably positive for cytokeratin.

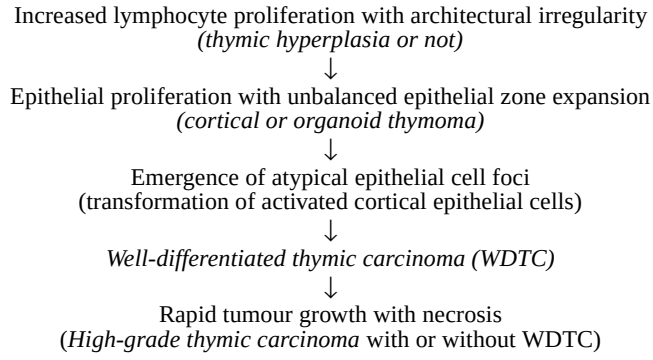


Fig. 13 Sequence of thymic lesions in SV40T transgenic mice

On staining with monoclonal antibodies (Th-3 and Th-4), both cortical and medullary epithelial cell markers were negative in thymic carcinoma; nor did any other antibody for thymic medullary epithelium (Biosource, MS-017-SN) show any reaction. The cortical epithelial cell marker, Th-3, was diffusely expressed in the epithelial cells of cortical-type thymoma; Th-4 was focally expressed in cortical-type thymomas. These results indicated that thymomas in these mice were of cortical origin, and also that the original immunophenotype changed on progression to thymic carcinoma.

Discussion

In laboratory animals, spontaneously occurring thymic epithelial cell tumours are uncommon [22, 27, 31, 51]. Both natural and experimentally induced thymic epithelial tumours have been used as models for dissecting the steps of intrathymic lymphocyte maturation [2, 22]. Thymic epithelial tumours similar to human spindle cell thymoma were produced by neonatal inoculation with polyoma virus in C3H/Bittner mice by Hoot and Kettman [17]. Only two reports of definite thymic epithelial tumours in TG mice were found, one with Thy1-myc TG mice [50] and the other with SV40T TG mice under the control of an erythroid specific enhancer [57]. Thymic hyperplasia has occasionally been encountered in TG mice harbouring SV40T antigen [3, 5, 7, 34, 36, 49], and Botteri et al. [3] described thymic hyperplasia in 19 of 33 founder mice at 5–14 weeks of age. Apart from being large, none of these hyperplastic thymuses showed architectural or cellular abnormality; prior to our series, thymic carcinoma in laboratory animals had never been observed. In our previous report [38], we described a stable line of TG mice which consistently developed thymic carcinoma after 23 weeks of age. We believed that this mouse model of thymic carcinoma could be utilized to determine the progressive steps of tumour development from thymoma to thymic carcinoma.

As we show here, there are sequential changes from cortical thymoma to thymic carcinoma via the step of WDTC. The thymuses of SV40T TG mice developed thymic carcinoma through a unique process, with a variety of histopathological changes, including an increase in the lymphoid cell population, epithelial zone expansion, multifocal malignant transformation of epithelial cells with organotypical findings, and large areas of thymic carcinoma with persisting pre-existing thymoma. Interestingly enough, these changes occurred in sequence. Although they relate to an animal model, our findings suggest that thymic carcinomas developed from cortical thymomas. Medullary thymomas did not develop in these mice, and no transition from medullary type thymomas to thymic carcinomas was observed.

The thymic carcinomas in our series were characterized by an admixture of thymoma elements in various proportions. The simultaneous presence of these two components in TG mice has not been described in the literature; there have, however, been a few case reports of human thymic carcinoma against a background of long-standing thymoma [35, 42, 48, 52], and Suster and Moran [54] recently studied 22 cases of primary thymic epithelial neoplasm showing combined features of thymoma and thymic carcinoma. They suggested that their cases supported the existence of a continuum in the spectrum of differentiation between thymoma and thymic carcinoma, suggesting a close histogenetic relationship between these two conditions [54]. Kuo et al. [23] described two cases of squamous cell carcinoma and undifferentiated carcinoma that were presumed to have developed from thymoma. Similar observations were made by Shimosato et al. [48] and by Morinaga et al. [35]. A recent report by Pescarmona et al. [42] suggested histological progression of thymoma following a study of recurrent cases, especially of cortical-type thymoma, including predominantly cortical thymoma, cortical thymoma, and WDTC. The above findings in humans, together with our data collected in an animal model, strongly suggest that thymic carcinoma may arise against the background of thymoma.

There are other data to support the belief that thymoma is a progressive disease, and that thymoma and thymic carcinoma form a continuum. The 5- and 10-year survival rates of patients with stage I thymoma have been reported to be 96.2% and 66.7%, respectively [30]. This result suggests that noninvasive thymoma may progress to invasive/metastatic thymoma if left untreated.

TG mice expressing the SV40T gene with its own regulatory region have been reported to develop choroid plexus tumours, thymic hypertrophy and kidney dysplasia [5]. These findings are comparable to ours. Renal abnormalities such as tubular dysplasia, polycystic change, adenoma of the renal tubules and sometimes carcinoma were consistently found in our series, although none of the animals showed choroid plexus tumour. Both Northern blot analysis and immunohistochemical staining of TG thymuses demonstrated SV40T expression in thymic epithelial cells in the whole experimental group. According to Moll et al. [34], neonatal GRF-Tag mice express SV40T specifically in a small subset of thymic epithelial cells that are highly enriched near the corticomedullary junction of thymic hyperplasia. In our series, however,

both most epithelial cells and thymic carcinoma cells in the whole experimental group expressed SV40T. Nevertheless, epithelial cells forming Hassall's corpuscle did not show SV40T staining. The finding of a diffuse expression pattern of SV40T on epithelial cells in early periods, which is also not confined to the corticomedullary junction, suggests that thymic lesions in periods I and II may be thymomas.

TG studies of other tumour types have led to the tentative conclusion that SV40 genes are not sufficient to induce tumours [7, 33, 59]. For SV40-induced choroid plexus tumours, this suggestion has stemmed from the observation of nonuniform SV40 large T antigen expression in focal tumours [33, 59]. According to Chen and van Dyke [7], differences in the rate of choroid plexus tumourigenesis reflect differences in the control regions of the two viruses, SV40 and lymphotropic papovavirus, rather than differences in T antigen per se. While SV40T expression under SV40 regulation was limited to a fraction of the choroid plexus cells prior to the formation of focal tumours, its expression under lymphotropic papovavirus control was generally uniform throughout the choroid plexus, with ensuing rapid tissue expansion [7]. In pancreatic beta cells, uniform expression of T antigen is not sufficient for the immediate induction of proliferation; several weeks are required before abnormal proliferation begins [56]. This result indicates an interesting difference in the ability of certain distinct cell types to respond to SV40T. Other testable possibilities include differences in T antigen levels in the state of cellular differentiation at the onset of T antigen expression [7]. Why does the SV40T gene specifically target thymus and renal tubules, in spite of using its own promoter and enhancer? We cannot explain this organ specificity. We speculate, however, that pancreatic islet cells, choroid plexus, thymic epithelial cells, and renal tubular cells are somewhat similar in that they all exhibit a varying degree of secretory function.

In SV40T TG mice, thymic tumours develop consistently, and among these tumours there appear a sequence leading from cortical thymoma to thymic carcinoma (Fig. 13). This SV40T model is in one particular animal, and when the conclusions are applied to humans an awareness of the many limitations and differences is essential. Nevertheless, these TG mice may provide an interesting model through which the progression from cortical thymoma to WDTC and high-grade carcinoma can be investigated.

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