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Recurrent Genetic Aberrations in Thymoma and Thymic Carcinoma

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Apart from single reported aberrant karyotypes, genetic alterations in thymic epithelial neoplasms have not been investigated so far. In this study, 12 World Health Organization classification type A thymomas (medullary thymomas), 16 type B3 thymomas (well-differentiated thymic carcinomas), and nine type C thymomas, all of them primary thymic squamous cell carcinomas, were analyzed by comparative genomic hybridization and fluorescence *in situ* hybridization. With the exception of one single case, type A thymomas did not reveal chromosomal gains or losses in comparative genomic hybridization. In contrast, all type B3 thymomas showed chromosomal imbalances, with gain of 1q, loss of chromosome 6, and loss of 13q occurring in 11 (69%), six (38%), and five (31%) of 16 cases, respectively. In primary thymic squamous cell carcinoma, the most frequent chromosomal losses were observed for 16q (six of nine cases, 67%), 6 (4 of 9, 44%), and 3p and 17p (three of nine each, 33%), whereas recurrent gains of chromosomal material were gains of 1q (5 of 9, 56%), 17q, and 18 (three of nine each, 33%). This study shows that the distinct histological thymoma types A and B3 exhibit distinct genetic phenotypes, whereas type B3 thymoma and primary thymic squamous cell carcinoma partially share genetic aberrations. In addition to the possible tumorigenic role, the deletion in type B3 thymoma of chromosome 6, harboring the HLA locus, might play a role in the pathogenesis of paraneoplastic autoimmunity characteristic of thymoma. *(Am J Pathol 2000, 157:257–266)*

Thymomas and thymic carcinomas are rare mediastinal neoplasms arising from thymic epithelial cells. In the new World Health Organization (WHO)[1] classification, several types of thymomas are distinguished based on histological criteria: 1) type A thymomas (also called medullary or spindle-cell thymoma); 2) type AB thymomas (also called mixed thymoma); 3) type B thymomas which are subclassified as type B1 thymomas (also called lymphocyte-rich thymoma, lymphocytic thymoma, predominantly cortical thymoma, or organoid thymoma), type B2 thymomas (also called cortical thymoma) and type B3 thymomas (also called epithelial, atypical, or squamoid thymoma or well-differentiated thymic carcinoma, respectively) and; 4) type C thymomas (thymic carcinomas) which exhibit morphological similarities to corresponding neoplasms in organs other than the thymus (eg, primary thymic squamous cell carcinoma). Although tumor stage has been shown to be the most important prognostic factor in thymoma,[2,3] the histological subtypes seem to be of independent prognostic significance.[4,5] Whereas type A and type AB thymomas frequently follow a benign clinical course, types B1–3 need to be considered as low to moderate malignant neoplasms.[6] Thymic carcinomas usually follow a highly malignant clinical course.[7,8]

An important aspect of thymoma biology is the occurrence of paraneoplastic autoimmune phenomena, which are frequently the presenting symptoms of these tumors.[9] In particular, myasthenia gravis commonly manifests in thymoma patients, the frequency depending on the histological type of thymoma.[10,11] Whereas much has been learned about the pathogenesis of paraneoplastic autoimmunity in thymoma,[12,13] little is known about the molecular mechanisms of thymoma oncogenesis. In particular, in contrast to many other solid tumors, the genetic background of thymoma remains unknown so far. There are only few case reports of aberrant thymoma and thymic carcinoma karyotypes published in the literature.[14–21] Apart from occasional reports of a recurrent translocation, t(15;19)(q15;p13), in an unusual, high-grade thymic carcinoma occurring in pediatric and young adult patients,[19–21] no recurrent genetic aberrations were shown.

In this study, we investigated the genetic aberrations of thymomas by comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH) in 37 cases of type A, type B3, and type C thymomas, all of them

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primary thymic squamous cell carcinomas. This study demonstrates that type A thymomas infrequently show genetic aberrations detectable by CGH, whereas type B3 thymomas and primary thymic squamous cell carcinomas are characterized by frequent and recurrent genetic changes. These aberrations might both help to understand the pathogenesis and interrelationship of these neoplasms and might further contribute to the understanding of pathogenetic mechanisms of paraneoplastic autoimmunity in thymomas.

Materials and Methods

Specimen Selection

Twelve cases of type A thymomas (medullary thymomas), 16 cases of type B3 thymomas (well-differentiated thymic carcinomas) and nine cases of type C thymomas, all of them primary thymic squamous cell carcinomas, were selected from the files of the Institute of Pathology at the University of Würzburg. All cases were independently reviewed by two of the authors (HKMH and AM) following previously published criteria.[1,4,11,22] To assure a sufficiently high content of neoplastic epithelial cells for subsequent CGH analysis (which requires a tumor cell content of >50%),[23] only cases with <40% lymphocytes were included into the series. In borderline cases, immunohistochemical stains for cytokeratin CK19, CD1, and CD3 were performed to control tumor cell content. This series did not include type AB, B1, and B2 thymomas because of the exceedingly high and diffuse admixture of lymphocytes in these tumors. In none of the thymic squamous cell carcinomas was there any evidence for a primary extrathymic malignancy with secondary spread to the thymus or of an additional type A, AB, or B1–3 thymoma component as occasionally reported.[24]

DNA Extraction

Before DNA extraction, a hematoxylin and eosin (H&E)-stained reference slide of each block was screened to decide if only a part or the whole material contained sufficient thymoma epithelial cells. A dissection of tumor tissue was carried out if the whole tissue was not suitable for analysis because of focal lymphocyte-rich areas.

In five of 12 type A, in 10 of 16 type B3 thymomas, and in four of nine primary thymic squamous cell carcinomas, fresh-frozen tissue material was available. DNA extraction was performed according to routine protocols using the phenol-chloroform extraction method.[25] Briefly, between 20 to 30 5-μm cryostat tissue sections were suspended for overnight digestion at 50°C in digestion buffer containing 100 mmol/L NaCl, 10 mmol/L Tris-HCl, pH8.0, 25 mmol/L ethylenediaminetetraacetic acid (EDTA), pH 8.0, and 0.5% sodium dodecyl sulfate (SDS) (all chemicals provided by Sigma, St. Louis, MO) with 0.1 mg/ml proteinase K (Roche Diagnostics, Mannheim, Germany). DNA was isolated by subsequent washes with phenol, Tris-HCl equilibrated phenol/chloroform, and chloroform, respectively. DNA was precipitated by addition of 1/10 volume of 3 mol/L sodium acetate and 1 ml of 100% cold ethanol at −70°C for 2 hours. After a wash with 70% cold ethanol, the DNA pellet was air-dried and resuspended in 0.5 mol/L TE buffer (10 mmol/L Tris-HCl, pH 7.6, 1 mmol/L EDTA).

In seven type A thymomas, 6 type B3 thymomas, and five thymic squamous cell carcinomas, only formalin-fixed material was available. Ten to 50 6-μm sections of paraffin-embedded tissue blocks were deparaffinized by two washes each with xylene and 100% ethanol, respectively, followed by drying of the material for 2 hours at 50°C. The completely dried tissue pellets were subsequently incubated for 12 to 24 hours in 1 mol/L NaSCN at room temperature. The tissue was then digested for 72 hours in a buffer containing 75 mmol/L NaCl, 0.5% Tween 20, 25 mmol/L EDTA, pH 8, and 600 μl/ml proteinase K at 56°C. After 24 and 48 hours, the digestion procedure was controlled; if necessary, proteinase K was added again. DNA was then isolated from the completely digested tissue material following the above described phenol-chloroform extraction procedure and ethanol/sodium acetate precipitation.

Comparative Genomic Hybridization

CGH was performed according to a standard protocol.[26] Briefly, tumor DNA was labeled by nick translation with biotin-16-dUTP (Roche Diagnostics, Mannheim, Germany). Reference DNA, extracted from lymphocytes from a healthy donor, was labeled with digoxigenin-11-dUTP (Roche Diagnostics). The amount of DNase I (Roche Diagnostics) and DNA polymerase I (Promega, Mannheim, Germany) were adjusted to obtain DNA fragment sizes of ~500 to 1000 bp. After inactivation of DNase I, unincorporated nucleotides were removed by gel filtration on Sephadex G50-packed columns (Sigma). Equal amounts of test and reference DNA (1 μg each), together with 70 μg of Cot-1-DNA for blocking repetitive sequences, were co-hybridized on commercially available metaphase slides (Vysis, Downers Grove, IL). Denaturation of the prewarmed slides before hybridization was performed with 70% deionized formamide/2× standard saline citrate (SSC)/50 mmol/L sodium phosphate, pH 7.0, for 3 to 5 minutes at 70°C in a water bath, followed by dehydration in cold ethanol. After overnight hybridization at 37°C, the slides were washed four times with 50% formamide/2× SSC, pH 7.0, at 42°C and three times at 60°C with 0.1× SSC. Detection of biotin and digoxigenin-labeled DNA probes was accomplished with fluorescein-isothiocyanate avidin (Vector Laboratories, Burlingame CA) or Cy3-conjugated anti-digoxigenin antibodies (Dianova, Hamburg, Germany), respectively. After detection, a 4,6-diamidino-2-phenylindole counterstain was performed for chromosome identification. The slides were then covered with antifade solution (Vectashields, Vector Laboratories).

Signals were visualized with a Zeiss Axiophot fluorescence microscope and analyzed with the ISIS digital image analysis system (MetaSystems, Altlussheim, Germany). At least 15 metaphases per case were analyzed.

Ratio values of 1.25 and 0.8 were used as upper and lower thresholds for identification of chromosomal gains or losses. A high-level amplification was defined as an overrepresentation of genetic material with the fluorescence ratio values exceeding 2.0 or based on the observation of strong focal signals in the fluorescein isothiocyanate fluorescence with the corresponding ratio profile being diagnostic for overrepresentation.

Fluorescence In Situ *Hybridization*

Fluorescence *in situ* hybridization was performed on cells isolated from the identical frozen tissue blocks used for DNA extraction according to previously published protocols.[27] Briefly, 10 to 20 10-μm cryostat tissue sections were minced with scissors and treated with C50T (0.05 mol/L citric acid monohydrate and 0.5% Tween 20) for 10 minutes. After washing in phosphate-buffered saline and centrifugation at 130 $\times$ *g* for 10 minutes at 4°C, the pellet was resuspended in −20°C cold Carnoy's solution. The cell suspension was dropped onto glass slides and dried overnight at room temperature.

After washing twice in 2× SSC, hybridization without previous digestion was performed. Biotin- or digoxigenin-labeled centromeric repetitive satellite DNA probes specific for chromosomes 1, 6, and 11 were purchased from Oncor (Gaithersburg, MD). They were used in double FISH experiments at concentrations of 1 ng of DNA probe in 10 μl of hybridization mixture containing 60% formamide, 2× SSC, 10% dextran sulfate, and 0.1% salmon sperm DNA according to the manufacturer's instructions. Simultaneous denaturation of the slides and the hybridization mixture containing the probe under a coverslip sealed with rubber cement was performed at 75°C for 5 minutes, followed by overnight hybridization at 37°C. After removing the coverslips, slides were washed three times for 5 minutes in 0.1× SSC at 60°C and subsequently in PN buffer (0.085 mol/L NaH_2PO_4, 0.085 mol/L Na_2HPO_4, 0.77 mol/L NaOH) for one minute at room temperature. Signal detection was accomplished using appropriate Cy3- and fluorescein isothiocyanate-conjugated antibodies (Roche Diagnostics). Visualization of the signals was performed with a Zeiss Axiophot fluorescence microscope. Illustrations were made using the ISIS imaging system (MetaSystems).

For signal evaluation, nuclear signals from at least 100 cells were analyzed per case. Generally, only slides with a good hybridization efficiency were evaluated. Nuclear signals had to be observed after hybridization in >80% of cells. Only cells with clearly discernible nonoverlapping nuclei were counted. Signals should have the same intensity, split signals were counted as one signal. Cut-off level was determined for the probes at 8% (mean ± 3SD) according to hybridization results in cases with no gains of 1q and losses of 6 in CGH.

Clinical Data

The series included 16 female and 21 male patients (Table 1). Patients' ages ranged from 57 to 74 years (average age, 66 years) in type A thymoma, from 29 to 73 years (average age, 55 years) in type B3 thymoma, and from 43 to 72 years (average age, 59 years) in thymic squamous cell carcinoma. For clinical stage according to the Masaoka staging system[28] and the frequency of paraneoplastic autoimmunity see Table 1.

In two cases, follow-up material of recurrent thymic neoplasia was available for CGH analysis: recurrent type B3 thymoma relapsing 2 years after initial diagnosis (case 13) and recurrent type C thymoma (squamous cell carcinoma) relapsing 4 years after initial diagnosis (case 30).

Results

Type A Thymoma (Medullary Thymoma)

Out of 12 type A thymomas, 11 cases did not show gains or losses of chromosomal material by CGH (Table 1 and Figure 1). In one case, however, a partial loss of the short arm of chromosome 6 was observed (rev ish dim(6)(p21-pter)).

Type B3 Thymoma (Well-Differentiated Thymic Carcinoma)

Sixteen cases of type B3 thymoma were analyzed by CGH, all of them showing gains or losses of chromosomal material (Table 1 and Figure 2). Gains (n = 20) and losses of chromosomal material (n = 15) occurred with overall similar frequencies. Recurrent gains of chromosomal material were gains of 1q (11 of 16 cases, 69%) and Xq (three of 16 cases, 19%). One case showed a high-level amplification of chromosomal band 8p12.

The most frequent recurrent losses were observed for the whole or parts of chromosome 6 (six of 16 cases, 38%) and for 13q (five of 16, 31%). In one case, recurrent thymoma could be compared with the initial lesion. Whereas gain of 1q and loss of chromosome 6 were detected in both specimens, additional gain of parts of chromosome 5 (5p, 5cen-q21) and X (Xq22-qter) were noted in the recurrent thymoma.

FISH analysis was performed with centromeric probes for chromosome 1 in four cases and for chromosome 6 in five cases of type B3 thymoma. In accordance with CGH data, FISH analysis revealed three to four signals with the centromeric probe of chromosome 1 in 19 to 72% of cells and one signal with the centromeric probe of chromosome 6 in 28 to 91% of cells in thymoma cases in which CGH had shown gains of 1q and loss of 6, respectively (Table 2 and Figure 3). FISH with centromeric probe of chromosome 11 showed diploidy in all cases analyzed.

Primary Thymic Squamous Cell Carcinoma (Type C Thymoma)

All nine cases of primary thymic squamous cell carcinoma showed genetic aberrations in CGH analysis (Table 1 and Figure 4). Altogether, there were 19 gains and 22 losses of chromosomal material. Recurrents gains

Table 1. Comparative Genomic Hybridization of Type A Thymoma (Medullary Thymoma), Type B3 Thymoma (Well-Differentiated Thymic Carcinoma), and Primary Thymic Squamous Cell Carcinoma: Patient Data and Chromosomal Gains and Losses Detected by CGH

Case	Age	Sex	WHO Type	Stage	MG	rev ish enh	rev ish dim
1	65	m	A	I	ng	—	—
2	66	m	A	I	yes	—	—
3	74	f	A	I	ng	—	—
4	60	f	A	I	no	—	—
5	73	f	A	II	no	—	—
6	57	f	A	I	ng	—	—
7	68	m	A	III	no	—	—
8	74	m	A	II	yes	—	−6p21-pter
9	65	m	A	I	no	—	—
10	71	m	A	I	ng	—	—
11	47	m	A	III	no	—	—
12	57	m	A	I	no	—	—
13	46	m	B3	IVb	yes	+1q	−6
	48	m	B3 R	IVb	yes	+1q, +5p, +5cen-q21, +Xq22-qter	−6
14	45	m	B3	III	yes	+1q22-qter, +Xq24-qter	−13q21-q31
15	63	f	B3	II	ng	+17q	—
16	65	m	B3	IVb	yes	+16	—
17	73	f	B3	II	yes	+1q	−6
18	42	f	B3	ng	yes	+1q	—
19	46	f	B3	III	yes	+1q, ampl 8p12	−6, −18q
20	63	m	B3	IVb	ng	+1q, +7	—
21	29	m	B3	II	yes	—	−13q
22	42	f	B3	II	yes	+1q	−6
23	53	f	B3	III	ng	—	−13q21-qter
24	61	m	B3	ng	ng	+1q	−6
25	47	f	B3	II	yes	+1q23-qter	—
26	63	m	B3	IV	no	+8, +Xq21-qter	−13q
27	57	m	B3	II	yes	+1q	−6q, −12p, −12cen-12q21
28	63	m	B3	III	yes	+1q	−3p, −3q13, −13q14-qter, −16q12-q21
29	ng	f	C(TSSC)	ng	ng	+5p, +8q	−3p13-pter, −8p, −5q15-q23
30	57	f	C(TSSC)	III	ng	—	−3
	61	f	C(TSSC) R	IVa	ng	—	−3
31	57	f	C(TSSC)	ng	ng	—	−6, −16q
32	45	m	C(TSSC)	III	no	+1q, +17q, +18q23	−16q21-qter, −17p13
33	67	m	C(TSSC)	III	no	+1q, +3q21-q22	−2q32-q33, −3p, −9, −13cen-q31, −16q, −17p13
34	63	m	C(TSSC)	II	no	+1q, +5, +18	−6, −13q, −16q
35	43	m	C(TSSC)	ng	no	—	−16q
36	64	f	C(TSSC)	III	no	+1q, +8, +17q, +18	−6, −16q, −17p
37	72	f	C(TSSC)	ng	no	+1q, +5q33, +14q22-qter, +15, +17q	−6q

Abbreviations: m, male; f, female; WHO type, type of thymoma according to the WHO classification [1]; TSSC, primary thymic squamous cell carcinoma; R, recurrent tumor; stage, stage of thymoma according to Masaoka[28]; MG, myasthenia gravis; ng, not given; rev ish enh, chromosomal gains detected by CGH; rev ish dim, chromosomal losses detected by CGH.

observed were gains of 1q (five of nine cases, 56%), and 17q and 18 (3 of nine cases each, 33%). Recurrent losses were observed for chromosomal material of 16q (six of nine cases, 67%), 6 (four of nine, 44%), and 3p and 17p (three of nine cases each, 33%).

In one case, recurrent primary thymic squamous cell carcinoma could be compared with the initial lesion. Both specimens consistently showed loss of chromosome 3.

FISH analysis was performed with centromeric probes for chromosomes 1 and 6 in two cases (cases 34 and 36; Table 2). In accordance with CGH data, FISH analysis revealed only one signal with the centromeric probe of chromosome 6 in 32 and 91% of cells, respectively. In case 36, FISH analysis with centromeric probes of chromosome 1 showed three to four signals in 14 and 15% of cells, respectively. In case 34, however, no additional signals were detected. In view of a clear CGH profile indicative of a gain of 1q in this case, this is probably attributable to a gain of 1q not involving the centromere.

Discussion

Genetic aberrations associated with thymic epithelial neoplasms are unknown so far. Currently, there are only very few reported aberrant karyotypes of thymoma and thymic carcinoma in the literature, which, in addition, were derived from different types of thymoma. Apart from occasional reports of a recurrent translocation t(15;19)(q15;p13) in an uncommon mediastinal neoplasm possibly related to thymic carcinoma and occurring in children and young adults,[19–21] no recurrent genetic aberrations

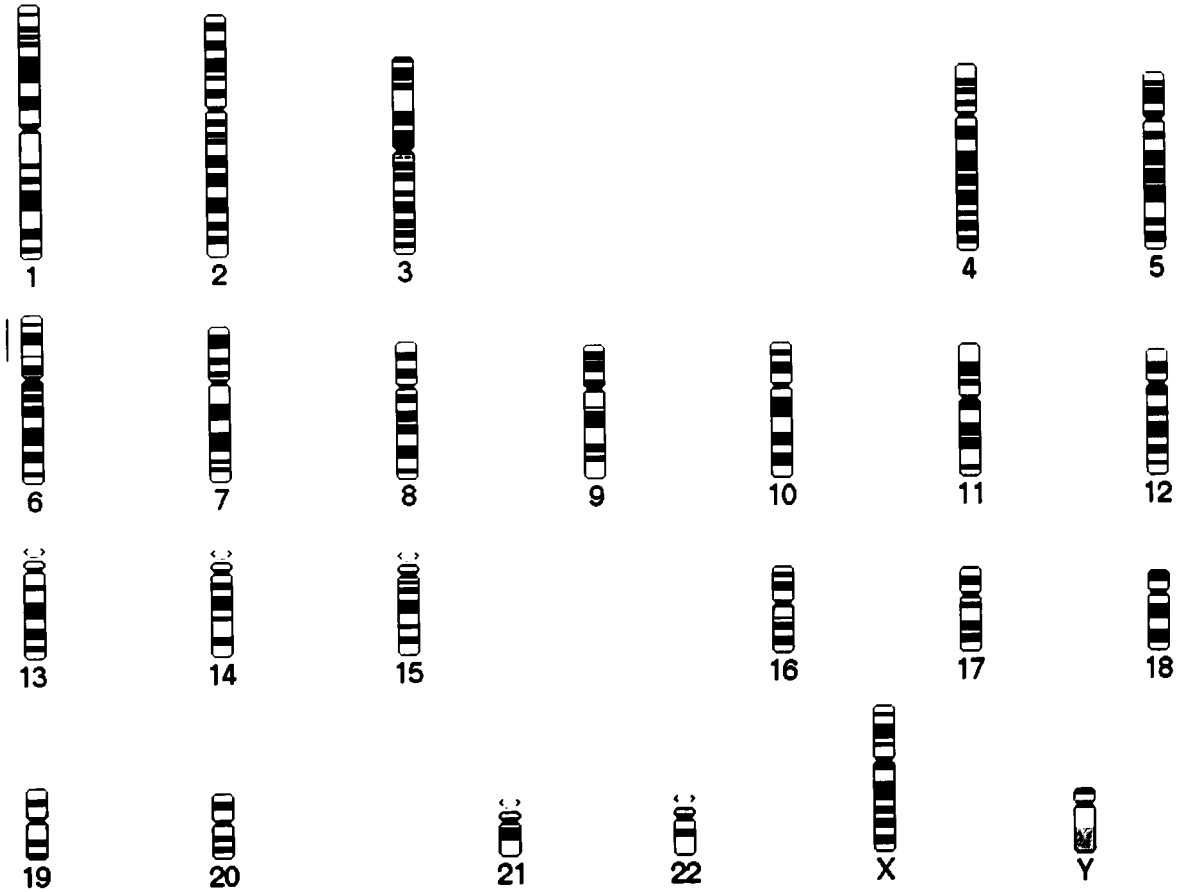

Figure 1. CGH analysis of type A thymoma (medullary thymoma). Out of 12 type A thymomas, only one case showed a chromosomal imbalance detectable by CGH (del 6p21-pter).

were found.[14–18] CGH has been shown to be a powerful tool in detecting chromosomal imbalances of solid tumors.[23,26] As far as thymomas are concerned, the diffuse admixture of numerous lymphocytes among neoplastic thymic epithelium in many thymomas, however, limits the application of CGH. We focused our study on cases of WHO type A, type B3, and type C thymomas with a high content of neoplastic epithelial cells to detect genetic aberrations potentially involved in thymomagenesis.

In type A thymoma, genetic aberrations detectable by CGH were found in only one of 12 cases. All other cases were devoid of chromosomal gains or losses. This finding correlates well with the usually benign clinical course of medullary thymoma.[4,5] Similar results have been obtained by CGH on other benign tumors.[29–32] The failure to detect chromosomal imbalances in most type A thymomas could be because of a lack of net DNA copy number changes in this thymoma type or because of small sizes of genetic imbalances not detectable by CGH. In addition, because CGH can only detect net gains or losses of chromosomal material, balanced genetic aberrations might be involved in the development of type A thymoma.

In contrast, all type B3 thymomas in our series revealed chromosomal imbalances by CGH. The most frequent gain of chromosomal material involved parts of, or the entire, 1q, which was detected in 68% of this thymoma entity, as confirmed by FISH analysis. Interestingly, Sonobe et al[18] reported an abnormal karyotype containing isochromosome 1q in addition to two normal copies of chromosome 1 in a case of "thymoma of the mixed lymphocytic and epithelial type." This tumor type most likely corresponds to WHO type B2 thymoma thought to be closely related to type B3 thymoma.[33] Gains of the entire long arm of chromosome 1 are frequently detected in a variety of human neoplasms.[34] Several potentially important growth promoting genes have been mapped to 1q.[35–42] Because all cases with 1q aberrations showed gains of large parts of 1q, no minimal overlapping region could be defined.

The second most frequent genetic aberration detected in type B3 thymoma was loss of chromosome 6. Interestingly, in two reported karyotypes of thymoma, deletions involving chromosome 6 have been reported—del(6)(q15) in a case of "cortical thymoma"[16] and ring chromosome r(6)(p2?2q2?) in a case of "benign encapsulated thymoma."[15] Loss of chromosome 6 may have two implications, one for thymomagenesis, the other for the immunological properties of thymomas.

First, both 6q and 6p are known to harbor potentially important tumor suppressor genes. Deletions of 6q are known to be among the most frequent chromosomal losses in human neoplasms.[43] Several chromosomal regions on 6q are assumed to harbor tumor suppressor

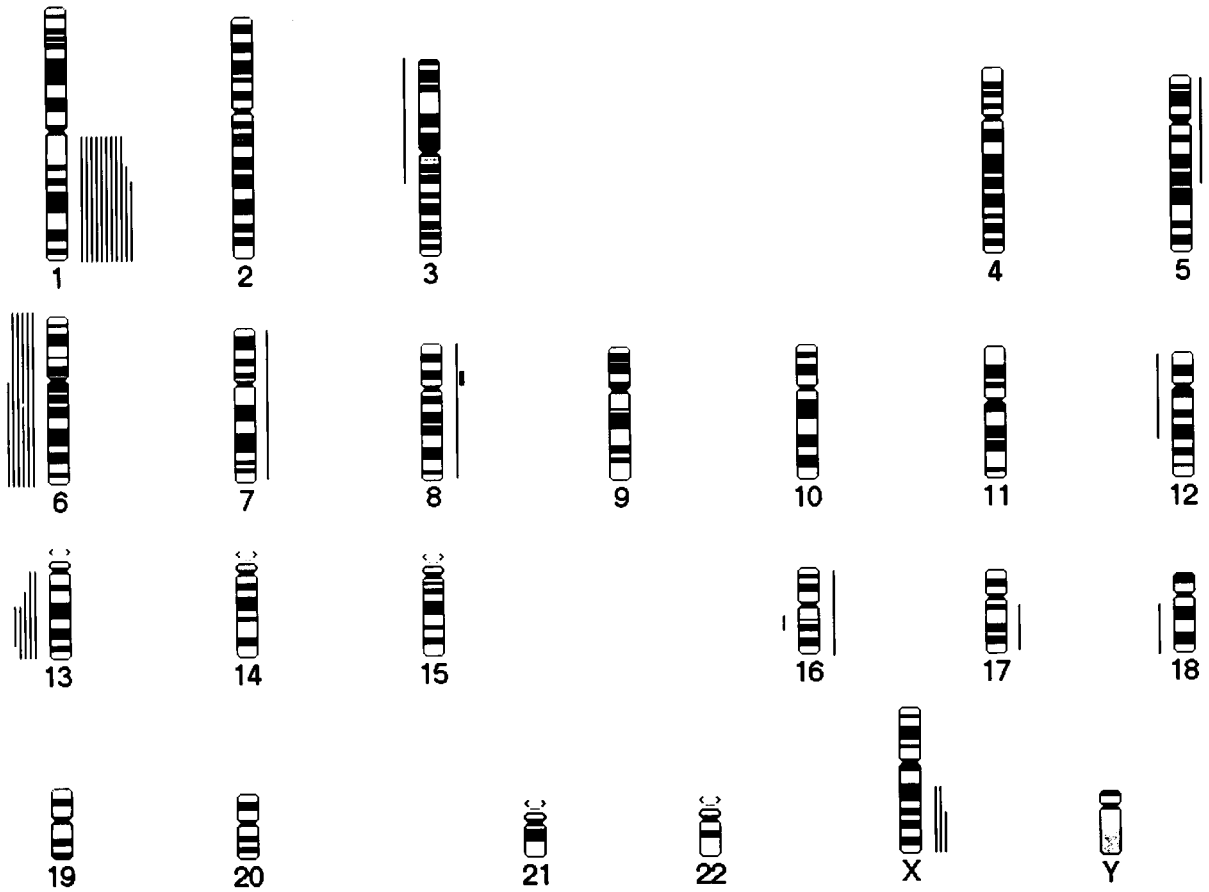

Figure 2. CGH analysis of type B3 thymoma (well-differentiated thymic carcinoma). All 16 type B3 thymomas showed chromosomal imbalances, with gain of 1q, loss of chromosome 6, and loss of 13q occurring in 11 (69%), six (38%), and five (31%) of 16 cases. **Bar** on the **left**: loss of chromosomal material; **bar** on the **right**: gain of chromosomal material. **Thick bar**: high-level amplification.

genes, eg, 6q21-q22.1 and 6q23.3-q25 in lymphoma,[44] the latter bearing the LOT-1/hZAC gene as a candidate tumor suppressor gene.[45] In contrast to deletions of 6q, deletions of 6p have only occasionally been reported as recurrent genetic aberrations in human neoplasms.[43] 6p harbors the cyclin-dependent kinase inhibitor 1A (p21/WAF/CDKN1A) at 6p21.2.[46]

Second, for thymoma, deletion of 6p is of major interest because of the presence of the human leucocyte antigen (HLA) locus on 6p21.3. Thymomas, in particular type B3 thymoma, are frequently associated with autoimmune phenomena.[10,11] The diversity of expressed HLA molecules on neoplastic thymic epithelial cells has not been investigated in detail so far. Because thymomas presum-

Table 2. Comparison of Data Obtained by Comparative Genomic Hybridization and Fluorescence *in Situ* Hybridization with Probes for Centromeric Regions of Chromosomes 1 and 6

Case	WHO type thymoma	CGH rev ish enh	CGH rev ish dim	FISH 1cen number of signals (percentage of cells)	FISH 6cen number of signals (percentage of cells)
8	A	—	−6p21-pter	not done	normal
13	B3	+1q	−6	4 (19%)	1 (40%)
13	B3, R	+1q, +5p, +5cen-q21, +Xq22-qter	−6	4 (72%)	1 (67%)
16	B3	+1q	−2	not done	normal
17	B3	+1q	−6	4 (50%)	1 (56%)
22	B3	+1q	−6	3 (42%), 4 (12%)	1 (28%)
34	C(TSCC)	+1q, +5, +18	−6, −13q, −16q	normal	1 (91%)
36	C(TSCC)	+1q, +8, +17q, +18	−6, −16q, −17p	3 (14%), 4 (15%)	1 (32%)

TSCC, primary thymic squamous cell carcinoma; FISH 1cen, FISH 6cen; fluorescence *in situ* hybridization with probes for centromeric region of chromosome 1 and 6, respectively; rev ish enh, chromosomal gains detected by CGH; rev ish dim, chromosomal losses detected by CGH. Analysis with centromeric probes of chromosome 11 showed diploidy in all cases examined.

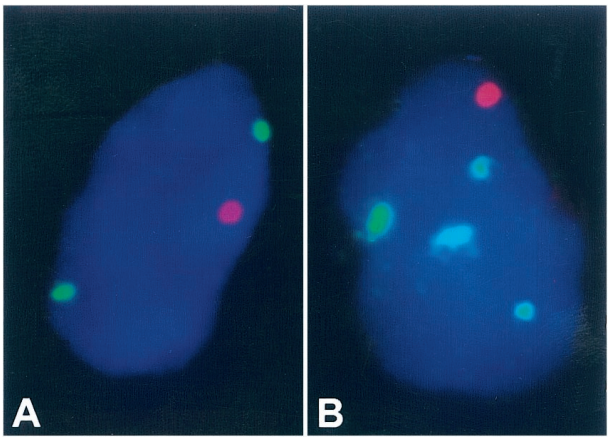

Figure 3. Fluorescence *in situ* hybridization of a WHO type B3 thymoma (case13). **A:** Green signals, centromeric probe for chromosome 11; red signal, centromeric probe for chromosome 6, corresponding to a loss of chromosome 6 observed in this case in CGH. **B:** Green signals, centromeric probe for chromosome 1; red signal, centromeric probe for chromosome 6, corresponding to a gain of 1q observed in this case in CGH in addition to a loss of chromosome 6.

ably are clonal neoplasms, the loss of chromosome 6 should involve the identical parental chromosome. Given the co-dominant expression of HLA genes, the diversity of expressed HLA haplotypes on neoplastic thymic epithelium in cases with monosomy 6 will be reduced. For certain autoimmune disorders such as rheumatoid arthritis and insulin-dependent diabetes mellitus, in addition to disease-favoring HLA haplotypes, HLA molecules protective of autoimmunity have been reported.[47,48] The pathogenesis of paraneoplastic autoimmune disorders in thymomas has been linked to the generation of T cells sensitized against various autoantigens.[9,12,13] In thymoma cases with deletion of chromosome 6, it may be speculated whether loss of protective HLA molecules on neoplastic epithelial cells might contribute to the formation of an autoaggressive T cell repertoire with subsequent development of autoimmune disorders.

Alternatively, monosomy 6 might lower the expression level of those HLA isotypes that are shared among the parental chromosomes, eliciting autoimmunity because of nontolerogenic T cell selection as reported in mice with reduced levels of HLA selectively on thymic epithelial cells.[49]

Another immunological consequence of reduced HLA diversity because of monosomy 6 could be the abnormal intratumorous selection of T cells exhibiting quasi-alloreactivity against HLA molecules that are lacking on the neoplastic thymic epithelial cells but occur outside the tumor. This hypothetical scenario resembles the presumed pathogenesis of myasthenia gravis after bone

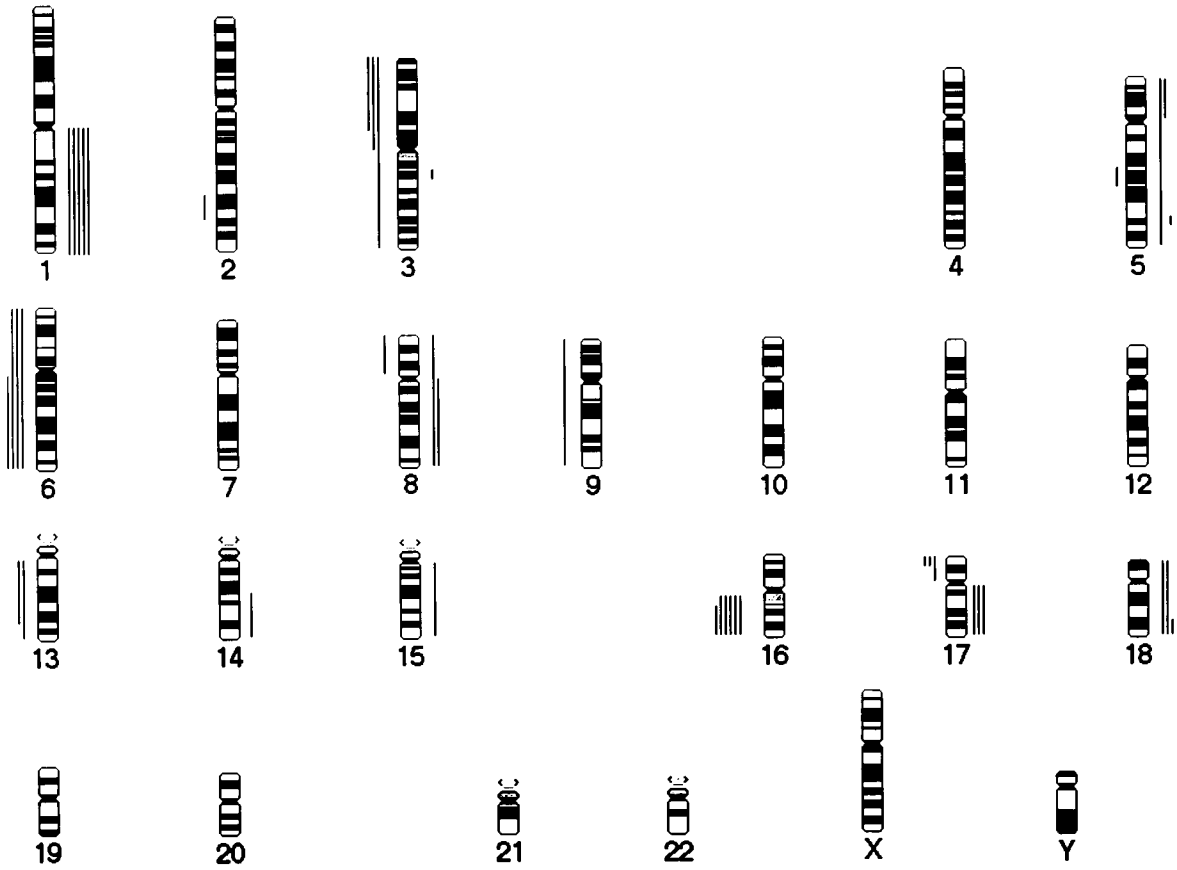

Figure 4. CGH analysis of primary thymic squamous cell carcinoma. In primary thymic squamous cell carcinoma, the most frequent chromosomal losses were observed for 16q (six of nine cases, 67%), 6 (four of nine, 44%), 3p and 17p (three of nine each, 33%), whereas recurrent gains of chromosomal material were gains of 1q (five of nine, 56%), 17q and 18 (three of nine each, 33%). **Bar** on the **left**: loss of chromosomal material; **bar** on the **right**, gain of chromosomal material.

marrow transplantation.[50] There is some evidence against the escape of anti-self HLA reactive T cells from thymomas to the periphery;[51] however, in that study, only two myasthenia gravis-associated thymomas were investigated, and none of them seems to have been a type B thymoma.

Deletion of the HLA locus may thus play a role in paraneoplastic autoimmunity in type B3 thymoma. Interestingly, the only case of type A thymoma in our series with a genetic aberration also showed a deletion involving the HLA locus; the patient suffered from myasthenia gravis that is uncommon in this thymoma entity. Clearly, further studies are necessary to investigate the role of HLA allele loss in thymoma-associated autoimmunity.

The third most frequent genetic imbalance detected in type B3 thymoma was loss of material of 13q in 33% of cases. 13q contains various tumor suppressor genes, among them RB1 and BRCA2.[52] Abnormal expression of RB1 based on immunohistochemistry has been reported in few cases of thymoma.[53]

In one case of type B3 thymoma, a high-level amplification was detected involving chromosomal band 8p12. This chromosomal region harbors a potential oncogene, namely the fibroblast growth factor receptor I, which has been reported to be amplified in other epithelial neoplasms, eg, in breast cancer and ovarian cancer.[54]

In contrast to type A, AB, and B thymomas, which are tumors unique to the thymus, type C thymomas make up a heterogeneous group of nonorganotypic carcinomas. These neoplasms are virtually indistinguishable from corresponding carcinomas arising in organs other than the thymus and are not associated with paraneoplastic autoimmunity.[11] In this series, we only included cases of primary thymic squamous cell carcinoma, as suggested by a characteristic tumor localization in the anterior mediastinum, absence of an extrathymic squamous cell carcinoma, characteristic histomorphology, and immunohistochemistry.[1]

The most frequent genetic imbalances detected in primary thymic squamous cell carcinoma were loss of 16q and—similar to type B3 thymoma—gains of 1q and loss of chromosome 6. 16q is known to harbor a variety of candidate tumor suppressor genes, among them RB2/p130,[55] CTCF,[56] the cadherin-encoding genes CDH1, CDH8, and CDH13,[57,58] and CMAR.[59] None of these genes has been implicated in the pathogenesis of primary thymic squamous cell carcinoma so far. However, Sonobe et al[17] recently reported a karyotype of a single case of thymic squamous cell carcinoma which showed two copies of der(16)t(1;16)(q12;q21.1) along with a structurally normal chromosome 16. These data suggest that genes on 16q might be involved in the pathogenesis of thymic squamous cell carcinoma. Of note, deletion of genes such as CMAR, and the cadherins, located on 16q, the most frequently deleted chromosomal regions in thymic squamous cell carcinoma, have been linked to the manifestation of invasive and metastasizing growth patterns in a variety of malignancies, eg, in breast cancer.[58,60]

Further recurrent losses noted in thymic squamous cell carcinoma were losses of 17p, involving the location of p53 at 17p13 and losses of 3p, harboring the putative tumor suppressor gene FHIT at 3p14.2, both of which have been reported to be involved in the oncogenesis of squamous cell carcinoma of other locations.[61,62]

The histogenetic relationship between type B3 thymoma and thymic squamous cell carcinoma has been a matter of debate; thymic squamous cell carcinomas have been reported to occasionally develop in thymomas, implying the possible clonal evolution of thymic carcinoma from thymoma.[24] Although none of our cases exhibited features of a combined thymoma, our data suggest that type B3 thymoma may in some instances be genetically related to thymic squamous cell carcinoma: in three cases of primary thymic squamous cell carcinoma, we found simultaneous gain of 1q and loss of chromosome 6, aberrations frequently detected in type B3 thymomas. In contrast, a relationship between type A thymoma and thymic squamous cell carcinoma seems less likely given the lack of shared recurrent genetic aberrations.

Although our study suggests that loss of chromosome 6, including the HLA locus, occurs as a recurrent genetic aberration both in WHO type B3 thymoma and primary thymic squamous cell carcinoma, the latter tumors are not associated with paraneoplastic autoimmunity.[11] In addition to loss of one HLA allele, further functional properties of neoplastic thymic epithelial cells required for the intratumorous generation of autoreactive T cells may have been lost in thymic squamous cell carcinoma.

In summary, our study for the first time shows that, similar to other epithelial neoplasms, distinct types of thymoma are associated with distinct, although not thymoma-specific recurrent chromosomal imbalances. Whereas type A thymomas, which usually are clinically benign, mostly are devoid of chromosomal gains or losses, usually malignant type B3 thymomas commonly show genetic aberrations, in particular involving 1q, chromosome 6, and 13q. Primary thymic squamous cell carcinoma frequently shows deletions of 16q and, in addition, may comprise neoplasms showing genetic relationship to type B3 thymoma.

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