Identical Allelic Losses in Mature Teratoma and Other Histologic Components of Malignant Mixed Germ Cell Tumors of the Testis

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Teratomas of the testis in post-pubertal patients are histologically diverse tumors that often coexist with other types of germ cell tumors. Using laser capture microdissection and loss of heterozygosity analysis, we investigated the clonality of mature teratoma and its relationship to other components of malignant mixed germ cell tumors to gain potential insight into the histogenetic relationship of teratoma with other germ cell tumor components. All 16 patients had mature teratoma as one component of their mixed germ cell tumors. The other histological subtypes included immature teratoma, seminoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma. Laser-assisted microdissection was performed on the formalin-fixed, paraffin-embedded tissue. Polymerase chain reaction was used to amplify genomic DNA at specific loci on chromosome 1p36.2 (D1S508), 2q22–32 (D2S156), 9p21–22 (D9S162), 11p13 (D11S903), 12q22–23 (D12S1051), and 18q21 (D18S46). Fourteen of 16 (88%) cases showed allelic loss in one or more components of the mixed germ cell tumors. Fourteen of 16 mature teratomas showed allelic loss in at least one of six microsatellite polymorphic markers analyzed. The frequency of allelic loss in mature teratoma was 50% (7 of 14) with D1S508, 33% (5 of 15) with D2S156, 58% (7 of 12) with D9S162, 43% (6 of 14) with D11S903, 20% (3 of 15) with D12S1051, and 33% (5 of 15) with D18S46. Completely concordant allelic loss patterns between mature teratoma and all of the other germ cell tumor components were seen in 10 of 14 tumors in which mature teratoma showed loss of heterozygosity. Our data support the common clonal origin of mature teratoma with other components of malignant mixed

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Germ cell tumor of the testis is the most common solid tumor malignancy in young adult men.[1] Studies show a six- to tenfold increase in germ cell tumors in patients with a first degree relative with germ cell tumor,[2] demonstrating a strong familial and genetic component. Mature teratoma is present in approximately 50% of mixed germ cell tumors (MGCT) but is uncommon as a "pure" germ cell tumor in post-pubertal patients, supporting the belief that teratoma is mostly derived from other forms of invasive germ cell tumor of the testis. Other components frequently seen in MGCT include immature teratoma, seminoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma.

Teratomas are histologically diverse tumors that contain a variety of tissues, including elements derived from ectoderm (squamous epithelium, neural tissue), mesoderm (muscle, cartilage, bone), and endoderm (intestinal, pancreatic, respiratory). Mature teratomas are usually composed of differentiated tissues from two or three of these germ cell layers. They occur in both children and adults, but the behavior in these groups is different, with the post-pubertal cases having associated metastases but not the pre-pubertal. Studies have shown that 22 to 43% of adult patients with pure teratoma may have metastases during their clinical course[3–5] and there is commonly coexisting intra-tubular germ cell neoplasia of the unclassified type in the testis.[3,6] The metastatic lesions in such cases may be comprised of either pure teratoma or other components of MGCT.[3,5] Regardless of whether these metastatic lesions are the direct metastasis of a primary teratoma or the teratomatous differentiation of a more primitive component after metastasis, it is evident that primary pure teratomas of the testis have metastatic potential, and should be considered malignant. These patients typically do well with complete surgical excision of the metastatic deposits.[7–9]
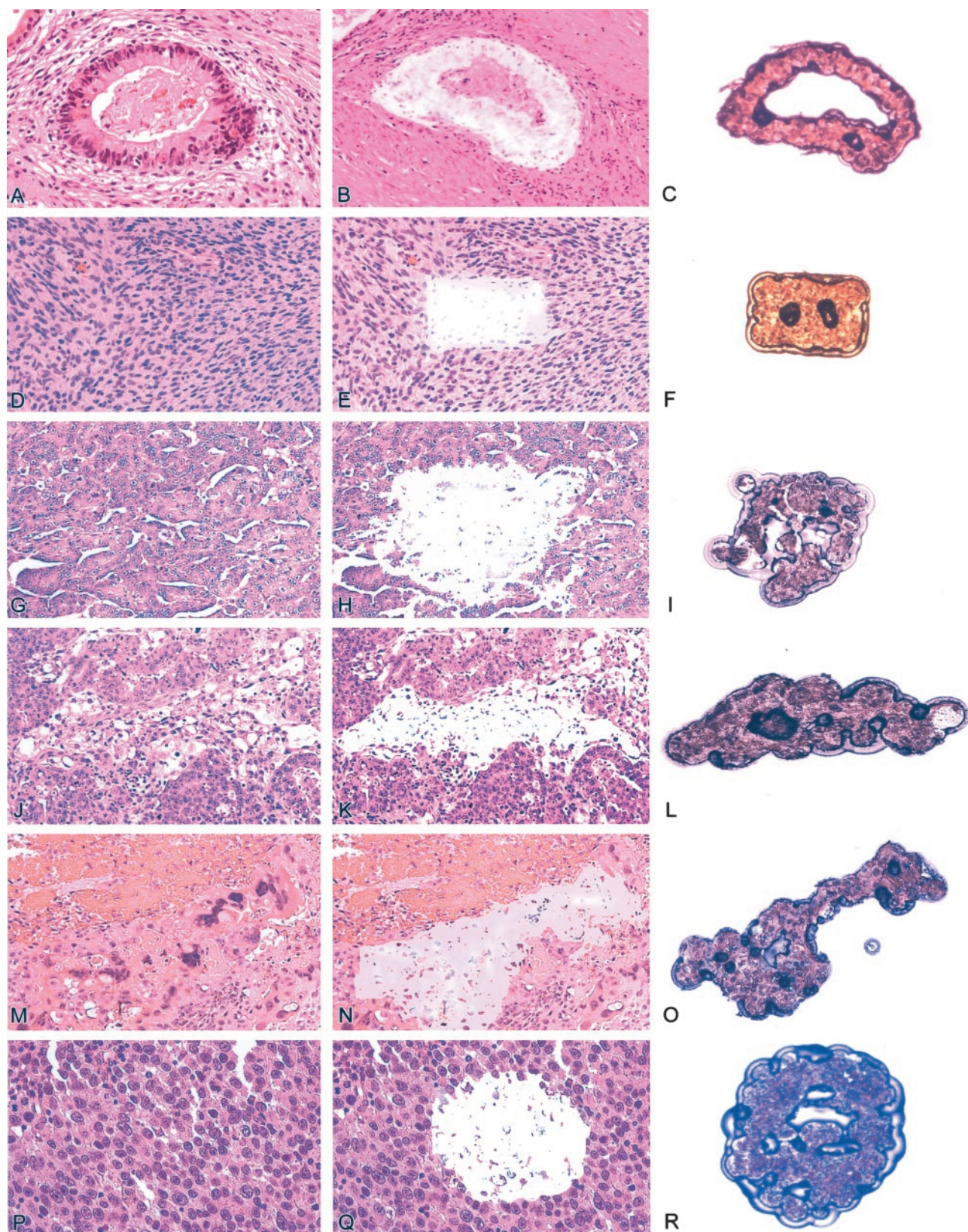
Figure 1. Laser micordissection of tumor from patients with malignant mixed germ cell tumor of the testis. Hematoxylin-eosin-stained sections showed mature teratoma (**A**), immature teratoma (**D**), embroynla carcinoma (**G**), yolk sac tumor (**J**), choriocarcinoma (**M**), and seminoma (**P**) before laser microdissection. **Panels B, E, H, K, N, G,** showed corresponding tumors after microdissection. **Panels C, F, I, L, O,** and **R** showed micordissected tissues ready for DNA analysis.

Table 1. Loss of Heterozygosity (LOH) Analysis of Malignant Mixed Germ Cell Tumors of the Testis

PATIENT	LOCUS	N	MT	IMT	EC	YST	SE	CC
1	D1S508	●	◖	◖	◖	◖		
	D2S156	●	●	●	●	●		
	D9S162	●	●	●	●	●		
	D11S903	⊘	⊘	⊘	⊘	⊘		
	D12S1051	●	◖	◖	◖	◖		
	D18S46	●	◖	◖	◖	◖		
2	D1S508	●	◖			◖		◖
	D2S156	⊘	⊘			⊘		⊘
	D9S162	●	●			●		●
	D11S903	●	◖			◖		◖
	D12S1051	●	●			●		●
	D18S46	●	◖			●		◖
3	D1S508	●	●		●	●	●	
	D2S156	●	●		⊘	●	⊘	
	D9S162	⊘	⊘		⊘	⊘	⊘	
	D11S903	●	●		●	●	●	
	D12S1051	●	●		●	●	●	
	D18S46	●	●		●	●		
4	D1S508	●	●	●	●			
	D2S156	●	◖	●	●			
	D9S162	●	◖	◖	◖			
	D11S903	●	◒	◒	◒			
	D12S1051	●	●	●	●			
	D18S46	●	◒	◒	◒			
5	D1S508	●	●		●			
	D2S156	●	●		●			
	D9S162	●	●		●			
	D11S903	●	◖		◖			
	D12S1051	●	●		●			
	D18S46	●	◒		◒			
6	D1S508	●	●	●			●	
	D2S156	●	●	●			●	
	D9S162	●	●	●			●	
	D11S903	●	●	●			●	
	D12S1051	●	●	●			●	
	D18S46	●	◖	◖			◖	
7	D1S508	●	◖	◖		◖		◖
	D2S156	●	●	●		●		●
	D9S162	●	◖	◖		◖		◖
	D11S903	●	◖	◖		◖		◖
	D12S1051	●	●	●		●		●
	D18S46	⊘	⊘	⊘		⊘		⊘
8	D1S508	●	◒	◒	◒	◒		
	D2S156	●	◒	◒	◒	◒		
	D9S162	⊘	⊘	⊘	⊘	⊘		
	D11S903	⊘	⊘	⊘	⊘	⊘		
	D12S1051	●	◖	◖	◖	◖		
	D18S46	●	●	●	●	●		
9	D1S508	●	●		●	●	●	
	D2S156	●	●		●	●	●	
	D9S162	●	●		●	●	●	
	D11S903	●	●		●	●	●	
	D12S1051	●	●		●	●	●	
	D18S46	●	●		●	●	●	
10	D1S508	●	◖	◖	◖	◖	◖	●
	D2S156	●	◖	◖	◖	◖	◖	◖
	D9S162	●	◖	◖	◖	◖	◖	◖
	D11S903	●	●	●	●	●	●	●
	D12S1051	●	◒	◒	◒	◒	◒	◒
	D18S46	●	●	●	●	●	●	●
11	D1S508	●	●		◖	◖	●	
	D2S156	●	●		●	●	●	
	D9S162	⊘	⊘		⊘	⊘	⊘	
	D11S903	●	●		●	●	●	
	D12S1051	●	●		●	●	●	
	D18S46	●	●		●	●	●	
12	D1S508	●	●	●	●	●		
	D2S156	●	●	●	●	●		
	D9S162	●	◖	◖	◖	◖		
	D11S903	●	◖	◖	◖	◖		
	D12S1051	⊘	⊘	⊘	⊘	⊘		
	D18S46	●	◖	◖	◖	◖		
13	D1S508	⊘	⊘		⊘			
	D2S156	●	●		●			
	D9S162	●	◖		●			
	D11S903	●	●		●			
	D12S1051	●	●		●			
	D18S46	●	●		●			
14	D1S508	●	●	●	●			
	D2S156	●	◖	◖	◖			
	D9S162	●	◖	◖	◖			
	D11S903	●	●	●	●			
	D12S1051	●	●	●	●			
	D18S46	●	●	●	●			
15	D1S508	●	◖				◖	
	D2S156	●	◖				◖	
	D9S162	⊘	⊘				⊘	
	D11S903	●	◖				◖	
	D12S1051	●	●				●	
	D18S46	●	●				●	
16	D1S508	⊘	⊘		⊘	⊘	⊘	
	D2S156	●	●		●	●	●	
	D9S162	●	◖		◖	●	◖	
	D11S903	●	●		●	●	●	
	D12S1051	●	●		●	●	●	
	D18S46	●	●		●	●	●	

N = Normal
MT = Mature Teratoma
IMT = Immature Teratoma
EC = Embryonal Carcinoma

YST = Yolk Sac Tumor
SE = Seminoma
CC = Choriocarcinoma

● = No allelic loss
◒ = Lower allele loss
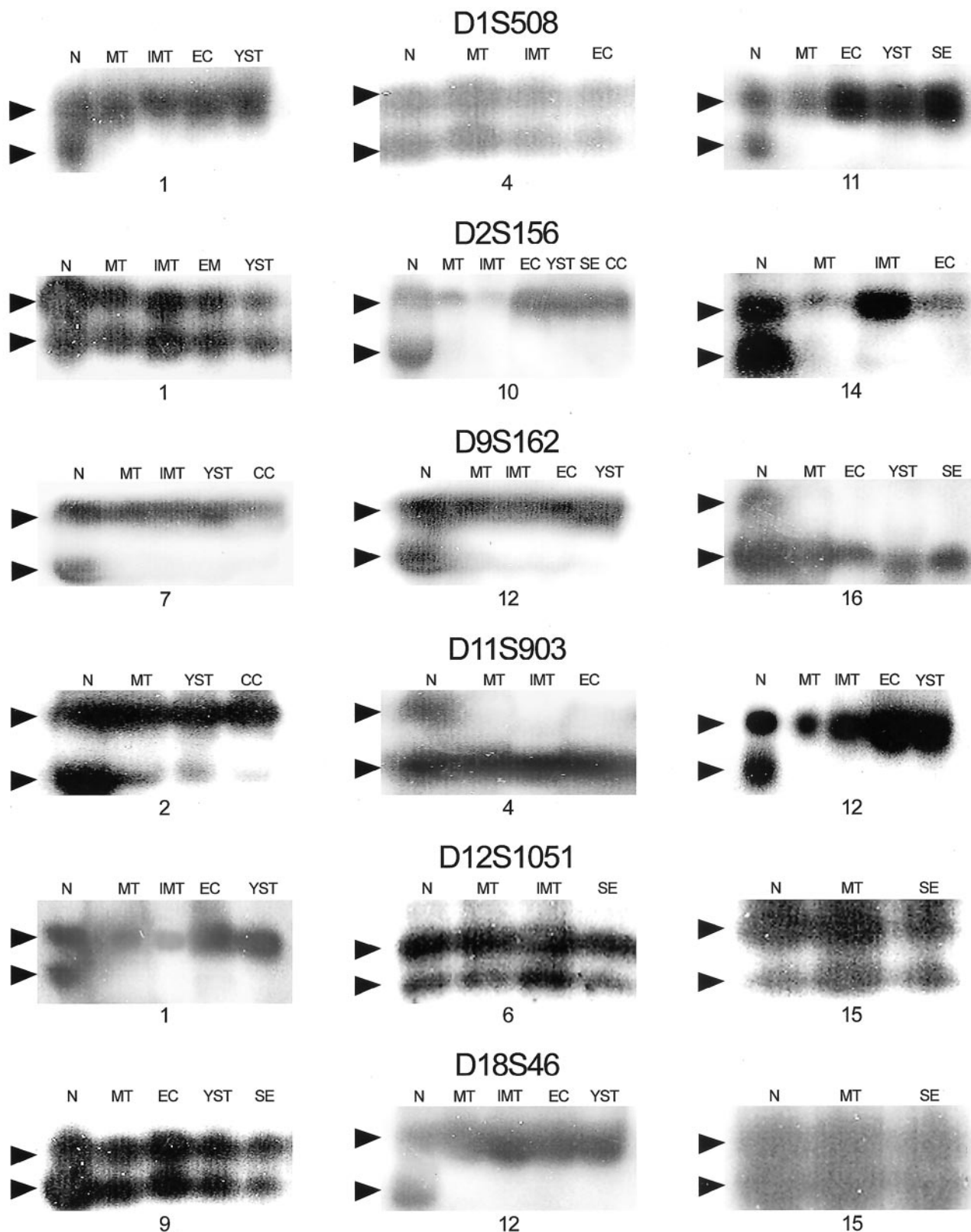◖ = Upper allele loss
⊘ = Non-informative

Figure 2.   Representative results of loss of heterozygosity analysis. DNA was prepared from normal tissue and different components of malignant mixed germ cell tumor, amplified by polymerase chain reaction using polymorphic markers D1S508, D2S156, D9S162, D11S903, D12S1051, and D18D46, and separated by gel electrophoresis. Arrows, allelic bands; *N*, normal tissue (control); MT, mature teratoma; IMT, immature teratoma; EC, embryonal carcinoma; YST, yolk sac tumor; CC choriocarcinoma, SE, seminoma.

Table 2. Overall Frequency of Loss of Heterozygosity (LOH) in Different Components of Malignant Mixed Germ Cell Tumors of the Testis

Locus	MT	IMT	EC	YST	SE	CC
D1S508	7/14 (50%)	4/8 (50%)	4/10 (40%)	6/9 (66%)	3/6 (50%)	2/3 (66%)
D2S156	5/15 (33%)	3/8 (38%)	3/12 (25%)	2/9 (22%)	2/7 (29%)	1/2 (50%)
D9S162	7/12 (58%)	5/7 (71%)	6/9 (66%)	3/7 (43%)	2/4 (50%)	2/3 (66%)
D11S903	6/14 (43%)	3/6 (50%)	3/10 (30%)	3/8 (38%)	1/7 (14%)	2/3 (66%)
D12S1051	3/15 (20%)	3/7 (43%)	3/10 (30%)	3/9 (33%)	1/7 (14%)	1/3 (33%)
D18S46	5/15 (33%)	4/7 (57%)	4/12 (33%)	2/9 (22%)	1/7 (14%)	1/2 (50%)

N, Normal; EC, Embryonal carcinoma; CC, Choriocarcinoma; MT, Mature teratoma; YST, Yolk sac tumor; IMT, Immature teratoma; SE, Seminoma.

The histogenesis of mature teratoma is uncertain. One hypothesis for the development of teratoma in adults, as mentioned, is differentiation from a non-teratomatous germ cell tumor element, a supposition which explains many of the aforementioned observations regarding teratoma; ie, the association with intra-tubular germ cell neoplasia, the uncommon occurrence of teratoma as a pure tumor, and the occurrence of non-teratomatous metastases in association with "pure" teratoma, which presumably is "pure" by virtue of spontaneous regression of its non-teratomatous precursor. It remains unclear, however, what components of germ cell tumors commonly give rise to teratoma, and there are even limited data regarding the common clonal origin of teratoma and other germ cell tumor components. We therefore investigated, through loss of heterozygosity studies, the relationship of teratoma and associated germ cell tumor components to gain insight into the histogenesis of teratoma.

Patients and Methods

Patients

Sixteen men with malignant MGCT of the testis who underwent radical orchiectomy in 2000–2001 were included. All patients had mature teratoma as one component of their germ cell tumor, as well as one or more other components of MGCT. The other components included immature teratoma, seminoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma. The patients ranged from 16 to 45 years of age, with a mean age of 26 years. The 2002 TNM (tumor, nodes, metastasis) system was used for pathological staging.[10] Nine were stage pT1, six were stage pT2, and one was stage pT3. This research was approved by the Indiana University Institutional Review Board.

Tissue Samples and Microdissection

Histological sections were prepared from formalin-fixed, paraffin-embedded tissue and were stained with hematoxylin and eosin for microscopic evaluation. From these slides, the various components of the mixed germ cell tumors were identified (Figure 1). Laser-assisted microdissection of the various components was performed on the unstained sections using a PixCell II Laser Capture Microdissection (LCM) system (Arcturus Engineering,

Mountain View, CA), as previously described.[11,12] Approximately 400 to 1000 cells of each component were microdissected from the 5-μm histological sections. Normal tissue from each case was microdissected as a control.

Amplification of DNA

The dissected cells were de-paraffinized with xylene and ethyl alcohol. Polymerase chain reaction (PCR) was used to amplify genomic DNA at various specific loci on chromosome 1p36.2(D1S508), 2q22–32(D2S156), 9p21–22(D9S162), 11p13(D11S903), 12q22–23(D12S1051), and 18q21(D18S46). Previous studies demonstrated that loss of heterozygosity (LOH) at these loci occur frequently in testicular germ cell tumors, particularly in teratomas.[8,13–21] Polymerase chain reaction amplification and gel electrophoresis were performed as previously described.[22–26] The criterion for allelic loss was complete or nearly complete absence of one allele in tumor DNA.[22–26] Polymerase chain reactions for each polymorphic microsatellite marker were repeated at least twice from the same DNA preparations and the same results were obtained. Results were reported as non-informative when visual inspection could not distinguish two distinct band forms in control DNA following polymerase chain reaction amplification.

Results

Fourteen of 16 (88%) patients with mixed germ cell tumors showed allelic loss in one or more components (Table 1). The number of specific loci lost ranged from one to four. Fourteen of 16 mature teratomas showed allelic loss in at least one of six microsatellite polymorphic markers. Figure 2 showed representative results of loss of heterozygosity analysis.

The frequency of allelic loss in mature teratoma was 50% (7 of 14) with D1S508, 33% (5 of 15) with D2S156, 58% (7 of 12) with D9S162, 43% (6 of 14) with D11S903, 20% (3 of 15) with D12S1051, and 33% (5 of 15) with D18S46. Table 2 shows the LOH rate among different components of MGCT (mature teratoma, immature teratoma, seminoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma).

Completely concordant allelic loss patterns between mature teratoma and the other germ cell tumor compo-

nents were seen in 10 of 14 tumors in which mature teratoma showed LOH. For example, cases 1, 7, 8, 12, and 15 demonstrated LOH at three separate alleles, all of which showed identical loss patterns in all tumor components present. In cases 7, 8, and 12, at least four different components of MGCT were present in the same tumor, and all showed identical allelic loss at all loci.

Only 4 of 14 cases (cases 2, 4, 10, and 16) showed some discordance between allelic loss of heterozygosity patterns. In case 2 (Table 1), the choriocarcinoma component showed allelic loss at locus D18S46, but none of the other components did, whereas in case 10, the choriocarcinoma component failed to show the same LOH that was seen in all other tumor components at locus D1S508, although identical allelic patterns were found at the other loci compared to the other components. In case 4, allelic loss was seen in the mature teratoma component at locus D2S156 that was not present in the immature teratoma or the embryonal carcinoma components. And in case 16, there was discordance in allelic loss at locus D9S162 in the yolk sac tumor component. In this case, the embryonal carcinoma and seminoma components shared identical allelic loss when compared with the mature teratomatous component.

Only two of 16 tumors (cases 3 and 9) did not show loss of heterozygosity in the mature teratoma component in at least one of the six loci. Both of these cases also lacked allelic loss in the other components of the MGCT at all six DNA loci.

Discussion

This study provides molecular evidence for the common clonal origin of mature teratoma with other components of malignant mixed germ cell tumors of the testis. Our data show complete concordance of allelic loss at six different chromosome microsatellite loci between mature teratoma and other germ cell tumor components in the majority of cases. These data supports that mature teratoma arises from the same progenitor cell as other components of MGCT and provides additional support for a pathogenetic model of adult teratoma of the testis that hypothesizes its usual derivation from other invasive germ cell tumor components.

Other recent evidence also supports a common clonal origin of MGCT. Rothe et al[19] analyzed 76 testicular germ cell tumors for LOH, of which 56 were pure tumors and 20 were comprised of two or more distinct germ cell tumor components. Of the 20 MGCT, 11 showed similar LOH patterns between the various components. A study using *in situ* hybridization for centromeric regions of chromosome 15 showed evidence of clonality among the different components of germ cell tumors, including the *in situ* and teratoma component.[27] de Graaff et al[28] separately karyotyped the two components, mature teratoma and choriocarcinoma, in a single case, and found that choriocarcinoma and mature teratoma showed almost identical patterns, with numerous complex chromosomal abnormalities. Mature teratoma and choriocarcinoma also showed a similar DNA aneuploid peak.[28] Previous stud-

ies also showed that teratoma was aneuploid, further supporting its malignant nature.[29]

The selective gain and loss of genetic materials is important in the development of testicular cancer.[30] Testicular mixed germ cell tumors likely result from activation of oncogenes and inactivation of tumor suppressor genes. The inactivation of tumor suppressor genes is demonstrated by LOH at specific loci.[19] A common chromosomal abnormality is gain of 12p, most often in the form of isochromosome 12p.[30–32] The gain of chromosomal material (oncogenes) and loss of tumor suppressor genes, demonstrated by loss of heterozygosity,[18,32] represent a multi-step process which leads to malignant transformation and cancer progression. LOH of several DNA loci is frequent in testicular germ cell tumors, including chromosome 2q, 11p, 12p, and 18q.[30] In addition, transformation to somatic-type malignancies have been shown in metastatic mature teratomas.[33]

Studies show that at 18q21.3, the *DCC* gene (deleted in colon cancer), is frequently lost in testicular germ cell tumors. Allelic loss of this chromosome region occurs in all histological subsets of germ cell tumors and may represent early genetic alterations in the development of germ cell tumors.[18] Peng et al[18] demonstrated LOH at 18q21.3 in up to 55% of informative cases. We found LOH in 33% of informative cases at this locus. LOH has also frequently been described at chromosome 11p13,[18,33,34] which appears to be in close proximity to the *WT*-1 gene. Previous studies showed LOH at this locus in 27% of informative cases of germ cell tumors.[18] Our study showed a 43% LOH at this locus.

van Echten et al[8] studied 12 patients with primary non-seminomatous germ cell tumors and post-chemotherapy residual mature teratoma and found several chromosomal abnormalities that differed between residual mature teratoma after chemotherapy and the original primary non-seminomatous germ cell tumors. However, in a subsequent larger study, the primary non-seminomatous germ cell tumors and residual mature teratoma showed comparable patterns of chromosomal abnormalities.[13]

In the current study, the number of components present in addition to mature teratoma ranged from one to five (immature teratoma, embryonal carcinoma, yolk sac tumor, seminoma, and choriocarcinoma). We found complete concordance of allelic loss in 10 of 14 cases when comparing mature teratoma with all other coexistent components of MGCT at six specific DNA loci used for this study. Of the 14 cases, only four showed discordance of allelic loss (cases 2, 4, 10, and 16) (Table 1). In case 2, the choriocarcinoma component showed allelic loss at locus D18S46 that was not present in the mature teratoma or associated yolk sac tumor components, although all other loci in these three components had identical patterns. In case 4, allelic loss was seen in the mature teratoma component at locus D2S156 that was not present in the immature teratoma or the embryonal carcinoma components. However, concordance of allelic loss was present in loci D9S162, D11S903, and D18S46. In case 10, there was only one component at one locus that differed from the allelic loss seen with the mature

teratoma. This was seen in the D1S508 locus of the choriocarcinoma component. Other than this component, there was complete concordance of allelic loss in the loci (D1S508, D2S156, D9S162, and D12S1051) involving all components of MGCT, including mature teratoma, immature teratoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma. In case 16, there was discordance in allelic loss only with locus D9S162 (yolk sac tumor). The embryonal carcinoma and seminoma components shared identical allelic loss of this locus with the mature teratomatous component.

It is interesting that in the four cases with some discordance, identical allelic loss was still found at other DNA loci. It is possible that the admixture of a small amount of normal tissue with the malignant cells of interest may contribute to the bi-allelic patterns seen in some tumor components. Tumor subtypes may have an integral, non-neoplastic stromal component that contributes to such falsely negative results.

The histogenesis of adult male germ cell tumor is complex. It is thought that adult male germ cell tumors arise from a precursor lesion, intra-tubular germ cell neoplasia, unclassified (IGCNU). It is felt that most often IGCNU gives rise to seminoma, which can progress to other components of MGCT, and ultimately mature teratoma.[34] Rarely, the intra-tubular component may progress directly to various germ cell tumor types such as yolk sac tumor and embryonal carcinoma. Our study sought specifically to address the question of a common clonal origin of mature teratoma with other components of MGCT. Although this study was not specifically designed to address the histogenesis of MGCT, the close relationship of LOH patterns in teratoma with other components of MGCTs is consistent with the derivation of teratoma from either a common progenitor cell or, further down the pathway, from an invasive malignant germ cell tumor. This would account for the similar LOH patterns in mature teratoma and the other components, as well as explain why mature teratoma is uncommon as a pure tumor in the post-pubertal testis. This would also explain the common association of mature teratoma with intra-tubular germ cell neoplasia, and the frequent occurrence of non-teratomatous metastases with pure mature teratoma of the adult testis. Presumably such cases represent tumors with regression of the non-teratomatous component in the testis. To specifically address the question of the MGCT histogenesis, future studies could look specifically at the LOH patterns of IGCNU and seminoma, and the relationship to other components of MGCTs. Our data support the common clonal origin of mature teratoma with other components of a mixed germ cell tumor in the majority of testicular cancer patients.

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