

# Specificity of *TLS-CHOP* Rearrangement for Classic Myxoid/Round Cell Liposarcoma

## *Absence in Predominantly Myxoid Well-Differentiated Liposarcomas*

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Myxoid liposarcoma (LS), the most common subtype of LS, is known to be characterized by the specific t(12;16) resulting in a *TLS-CHOP* fusion in almost all cases. We wished to address the following questions: (i) Is this genetic hallmark also present in other types of LS with predominant myxoid change? (ii) What is the proportion of cases with the variant *EWS-CHOP* fusion? (iii) What is the optimal approach for Southern blot detection of *TLS* breakpoints? We identified 59 LS characterized histologically by >90% myxoid component, in which frozen tissue tumor was available for DNA extraction. These 59 LS with myxoid features were divided into 2 groups: 42 LS with classic myxoid/round cell appearance (myxoid LS) and 17 well-differentiated LS (WDLS) with a predominant (>90%) myxoid component. Within the myxoid LS group, 29 tumors were low grade and 13 high grade (>20% round cell component). Among the 17 predominantly myxoid WDLS, there were 15 low grade and 2 focally high grade tumors. In addition, we selected as control group, 20 LS of other histological types with minimal or no myxoid change (17 WDLS and 3 pleomorphic LS) and 13 myxofibrosarcomas. Southern blot analysis was performed in all cases using a *CHOP* cDNA probe, and in all *CHOP* rearranged cases using a *TLS* cDNA probe. Probe/enzyme combinations for Southern blot analysis were *CHOP* exon 3–4 cDNA probe with *Bam*HI or *Sac*I, *TLS* exon 3–6 cDNA probe with *Bcl*II. All 42 cases of myxoid LS showed a *CHOP* rearrangement and 38 of them also had a *TLS* rearrangement. Among the 4 myxoid LS without Southern blot evidence of *TLS* rearrangement, 1 showed an *EWS-CHOP* fusion by Southern

blotting and reverse transcriptase-polymerase chain reaction and in another case, reverse transcriptase-polymerase chain reaction detected a *TLS-CHOP* fusion transcript. None of the predominantly myxoid WDLS and none of the tumors included in the control group showed rearrangements with *CHOP* probe. In addition, 12 predominantly myxoid WDLS, 10 other LS, and 5 myxofibrosarcoma from the control group were also tested for *TLS* rearrangement; all were negative. The *TLS-CHOP* fusion is highly sensitive and specific for the entity of classic myxoid/round cell LS. Other types of LS, even with a predominant myxoid component, lack the *TLS-CHOP* rearrangement, confirming that they represent a genetically distinct group of LS. The prevalence of the *EWS-CHOP* variant fusion was approximately 2% in this series. The optimal enzyme for *TLS* genomic breakpoint detection is *Bcl*II. (*J Mol Diag* 2000, 2:132–138)

Myxoid liposarcoma (LS) is the most common subtype of LS and occurs predominantly in the extremities.<sup>1,2</sup> A subset of cases show histological progression to round cell histology, which is associated with a significantly poorer prognosis.<sup>2</sup> The karyotypic hallmark of myxoid LS is the t(12;16)(q13;p11), present cytogenetically in >90% of the cases.<sup>3</sup> The translocation leads to the fusion of the *CHOP* and *TLS* genes at 12q13 and 16p11, respectively, and the generation of a *TLS-CHOP* hybrid protein.<sup>4–6</sup> In 4 cases of myxoid LS, a variant chromosomal translocation has been described, t(12;22), in which *CHOP* fuses instead with *EWS*, a gene highly related to *TLS*.<sup>7,8</sup>

Different cytogenetic features and their underlying molecular alterations define distinct entities among LS. A strong and specific association of the t(12;16) with myx-

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oid LS has been confirmed by most cytogenetic and molecular analyses.<sup>4,9-13</sup> The same translocation is present in pure round cell LS and combined myxoid and round cell LS, confirming the biological continuum between these two forms of LS, proposed on histopathological grounds.<sup>9,11-13</sup> In contrast, well-differentiated LS (WDLS) and pleomorphic LS contain no specific recurrent translocation. Instead, WDLS are characterized by cytogenetic evidence of gene amplification (giant marker chromosomes, ring chromosomes, double minutes), whereas pleomorphic LS typically show highly complex karyotypes.<sup>10,14,15</sup> Nonetheless, a single recent report has suggested that *TLS-CHOP* fusion transcripts may also be present in pleomorphic LS and WDLS.<sup>16</sup> The existence of a mixed tumor with combined features of myxoid LS and WDLS has been proposed based on cases of LS showing histological features of both.<sup>11</sup> For instance, up to one-third of WDLS show some degree of myxoid change.<sup>15</sup> Furthermore, rare cases of myxoid LS have shown apparent dedifferentiation, a phenomenon typically associated with WDLS.<sup>17</sup> These problematic tumors showing combined features of myxoid LS and WDLS, most often seen in the retroperitoneum, have not been systematically studied for the presence of the t(12;16). Thus the issue of the histological specificity of the *TLS-CHOP* fusion and the broader question of the biological relationship among different forms of LS have not been completely resolved. Aside from these biological and nosological questions, the differential diagnosis of myxoid LS can be sometimes quite difficult, due to overlapping morphological features with other myxoid neoplasms, myxofibrosarcoma in particular.

In the present study, we sought to address these issues by systematically studying myxoid LS, WDLS, pleomorphic LS, WDLS with extensive myxoid change, and myxofibrosarcomas for the *TLS-CHOP* rearrangement by Southern blotting. The *TLS* genomic breaks are clustered in introns 5 and 7,<sup>18,19</sup> whereas the vast majority of *CHOP* breakpoints occur in intron 1 or immediately upstream of exon 1;<sup>19</sup> in both genes, the breakpoint regions are relatively small (<10 kb), allowing Southern blot analysis. We have therefore also evaluated the optimal approach for Southern blot detection of *TLS* breakpoints. As part of this study, we also provide the first estimate of the prevalence of the variant *EWS-CHOP* fusion in a large group of myxoid LS, a datum germane to the interpretation of *TLS-CHOP* molecular diagnostic assays in myxoid LS.

## Materials and Methods

### Study Group and Histopathological Data

We identified 59 LS characterized histologically by >90% myxoid areas (including areas of round cell change), operated at Memorial Sloan-Kettering Cancer Center (MSKCC) between 1987 and 1998, in which frozen tumor was available for DNA extraction. Patient materials were procured under MSKCC protocol 90-049 approved by our Institutional Review Board. The 59 LS with myxoid features were divided into two groups: 42 LS with classic

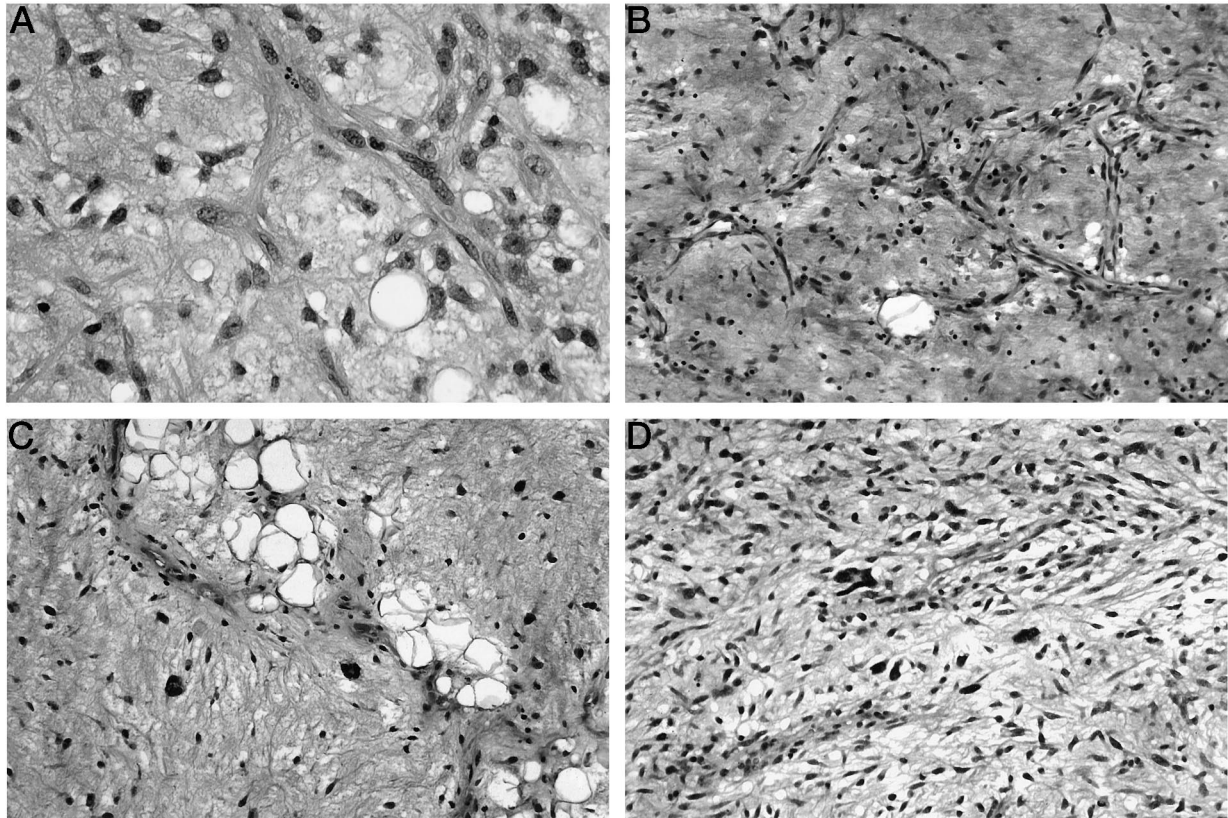
myxoid/round cell appearance (myxoid LS) and 17 well-differentiated LS with a predominant (>90%) myxoid change (predominantly myxoid WDLS). Within the myxoid LS group, 28 tumors arose in the extremities, 4 in the pelvic girdle, and 3 in the retroperitoneum, and 7 were multifocal. Histologically, 29 of the myxoid LS were low grade (Figure 1A) and 13 were high grade (defined as >20% round cell component). The predominantly myxoid WDLS tumors were located in the following axial sites: retroperitoneum (13 cases), pelvis (2 cases), intraabdominal (1 case), and mediastinum (1 case). No cases of this histological type presented in the extremities. The majority of predominantly myxoid WDLS were histologically low grade (15 cases), showing focal areas (by the study criteria <10%) of lipoma-like or sclerosing LS, diagnostic of WDLS (Figure 1B). In the remaining 2 cases of predominantly myxoid WDLS areas of dedifferentiation into a high grade spindle cell sarcoma were intermixed with the myxoid component.

In addition, we selected a control group of 20 LS of other histological types (17 WDLS and 3 pleomorphic LS) and 13 myxofibrosarcomas for which adequate frozen tumor was available for molecular analysis. The 17 cases of WDLS included in the control group showed lesser or absent myxoid change, as follows: 6 cases with 25 to 50% myxoid change, 5 cases with <25%, and 6 cases with no myxoid change (Figure 1C). Of these 17 WDLS, 13 were uniformly histologically low grade and 4 were focally high grade, and most were located in the retroperitoneum (15 cases). The other 2 WDLS were in the lower extremity. In addition to the WDLS, 3 cases of high grade pleomorphic LS of the extremities were also available for molecular analysis.

As the main differential diagnosis of myxoid LS in the extremities is with myxofibrosarcoma, we also included 13 cases of this tumor type in the control group (Figure 1D) to assess whether some cases in this morphological category are genetically related to myxoid LS. There were 5 low grade and 8 high grade myxofibrosarcomas, of which 10 arose in the lower extremity and 3 in the shoulder/axillary area.

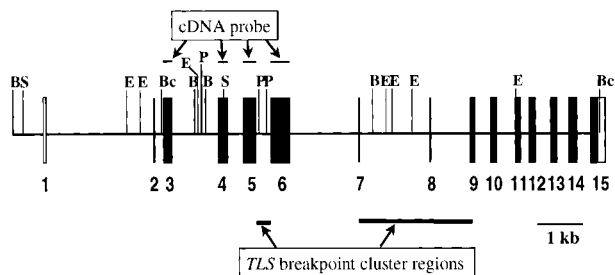
### Southern Blot Analysis

DNA was isolated from snap-frozen tumor tissue stored at -70°C using a standard organic extraction protocol. Southern blot analysis was performed on genomic DNA, digested in all cases with at least two restriction enzymes, separated by 0.7% agarose gel electrophoresis, transferred onto nylon membranes, and hybridized with radiolabeled probes. The *CHOP* and *TLS* probes were partial cDNA clones, derived from a full length *TLS-CHOP* cDNA (LPS41; gift of David Ron, New York University Medical Center, New York, NY).<sup>5</sup> The *CHOP* probe consisted of a 753-bp *PstI-XhoI* fragment corresponding to exons 3 and 4. This probe is expected to detect all *CHOP* rearrangements in genomic DNA digested with *BamHI* or *SacI*.<sup>4,20</sup> Extra bands, probably cross-hybridizing bands, were occasionally observed in samples digested with *SacI* and probed with the *CHOP* probe, and migrated at 5.5 and



**Figure 1. A:** Classic myxoid LS, low grade, with small, uniform tumor cells in a background of myxoid stroma and delicate “chicken-wire” type vascular network. Of note is the absence of the pleomorphic giant tumor cells characteristic of WDLS with myxoid changes. **B:** WDLS, predominantly myxoid. Although the myxoid background and the branching vasculature might suggest the diagnosis of classic myxoid LS, the tumor cells are larger and less uniform. **C:** WDLS, predominantly myxoid, with focal areas of lipoma-like LS and scattered tumor giant cells. **D:** Myxofibrosarcoma, low grade. Most of the tumor cells are uniform, but predominantly spindly, in contrast with the round to oval appearance of myxoid LS. Rare pleomorphic tumor cells are scattered within the myxoid stroma, which helps in distinguishing this tumor from classic myxoid LS.

3.5 kb. The *TLS* cDNA probe was a 780 bp *Xba*I-*Bgl*II fragment including exons 1 to 6 of *TLS*. According to the restriction enzyme site analysis of the complete genomic sequence of *TLS* (GenBank no. AF071213), this probe covers the entire *TLS* breakpoint region in *Bcl*I-digested DNA (Figure 2). Although the *TLS* cDNA fragment used as a probe included exons 1 and 2, these span only 90 and 25 bases, respectively, and are therefore too short to hybridize reliably with the corresponding restriction frag-



**Figure 2.** *TLS* genomic map with selected restriction enzyme sites (B, *Bam*HI; E, *Eco*RI; Bc, *Bcl*I; P, *Pst*I; S, *Sac*I). The map is based on the complete genomic sequence of *TLS* (GenBank no. AF071213, Ref. 17). Exons encoding translated sequences are shown as filled boxes, and the 3' untranslated portion of exon 15 is shown as an empty box. The *TLS* breakpoint cluster regions are based on references 18 and 19. The *TLS* probe is described in the text (see Methods). Partial restriction maps of the *CHOP* gene have been previously published.<sup>4,6,12,20</sup>

ments (as confirmed by the lack of these germline fragments on Southern blots). The *TLS* probe used was thus effectively equivalent to an exon 3 to 6 cDNA fragment, as depicted in Figure 2. In certain cases, mainly where the primary restriction enzymes were uninformative, other enzymes were used, such as *Eco*RI and *Hind*III for *CHOP* and *Pst*I and *Bam*HI for *TLS* cDNA probes. No polymorphisms were observed with the *TLS* probe, but it was associated with a background of multiple weak cross-hybridizing bands in certain samples. The *EWS* probe was a previously described 741-bp partial cDNA probe which hybridizes to exons 6 to 12 of *EWS*, covering in *Eco*RI and *Hind*III-digested DNA the entire genomic breakpoint cluster region.<sup>21</sup>

## Results

### Classic Myxoid LS Group

Among 59 LS with >90% myxoid histology, 42 cases were classified as classic myxoid LS. All of these 42 myxoid LS showed a *CHOP* rearrangement, and in 38 of them (90%) *TLS* rearrangement was also demonstrated (Table 1). Regarding the *CHOP* analysis, in 35 cases (83%) the *CHOP* rearrangement was detected with both



**Table 1.** Summary of Results

Histologic group	CHOP						TLS			
	Overall result	Results by restriction digest					Overall result	Results by restriction digest		
		<i>Bam</i> HI and <i>Sac</i> I digests*	<i>Bam</i> HI	<i>Sac</i> I	<i>Hind</i> III	<i>Eco</i> RI		<i>Bcl</i> I	<i>Bam</i> HI	<i>Pst</i> I
Myxoid LS ( <i>n</i> = 42)	42 R	35 R	38 R 2 G	38 R 3 G	1 R	1 R	38/42 R <sup>†</sup>	32 R 5 G	3 R	1 R
Predominantly myxoid WDLS ( <i>n</i> = 17)	17 G	17 G	17 G	17 G	6 G	3 G	12 G	10 G 2 F	2 G	2 G
WDLS with minor or no myxoid areas ( <i>n</i> = 17)	17 G	14 G	17 G	14 G	8 G	4 G	9 G	2 G	6 G	2 G
Pleomorphic LS ( <i>n</i> = 3)	3 G	3 G	3 G	3 G	1 F	2 G	1 G	1 G	ND	ND
Myxofibrosarcoma ( <i>n</i> = 13)	13 G	12 G	13 G	12 G	7 G	4 G	5 G	4 G	ND	1 G

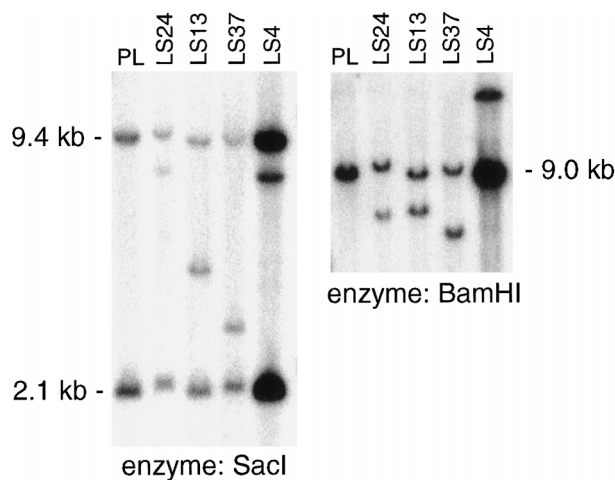
LS, liposarcoma; WDLS, well-differentiated LS; R, rearranged; G, germline; F, failure; ND, not done.

\*Except for this column, which shows the number of cases with identical results for both enzymes, in cells where the number of cases is less than *n*, the remaining cases were not studied with the enzyme in question, unless otherwise specified.

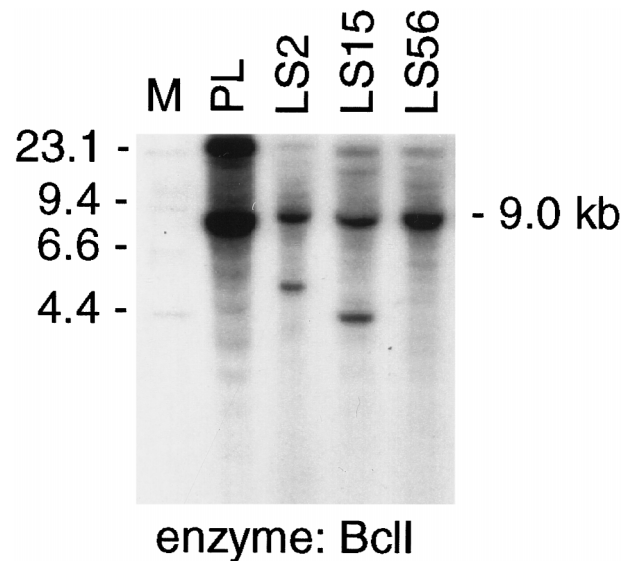
<sup>†</sup>Includes 2 cases that showed *TLS* rearrangement in *Eco*RI digests only; for details on 4 cases without detectable *TLS* rearrangement, see Results.

*Bam*HI and *Sac*I (Figure 3), in 3 cases only with *Bam*HI (germline pattern with *Sac*I), and in 3 cases only with *Sac*I (germline pattern in with *Bam*HI in 1 case and inadequate DNA for *Bam*HI digest in 2 cases). Finally, one case that was germline with *Bam*HI and had inadequate DNA for *Sac*I had shown rearrangements previously with two other restriction enzymes (*Eco*RI and *Hind*III). Regarding the detection of *TLS* rearrangements, 32 of 37 cases (86%) studied with *Bcl*I digests were positive (Figure 4). In 6 additional cases, *TLS* rearrangement was detected with other restriction enzymes, including *Eco*RI, *Bam*HI, and *Pst*I (see Table 1). Among the 4/42 cases that were not rearranged with *TLS* probe, 1 case could not be further tested due to degraded DNA, while 3 had sufficient DNA for further Southern blot analysis which showed a germline pattern with 4 additional restriction enzymes (*Bam*HI, *Sac*I, *Eco*RI, *Hind*III). Unfortunately, karyotypic data were not available in any of these 4 *TLS*-germline cases. One of the latter was tested for

*TLS-CHOP* by reverse transcriptase-polymerase chain reaction (RT-PCR) and found to have the relatively uncommon fusion of *TLS* exon 8 to *CHOP* exon 2 (results not shown). The remaining 2 cases of myxoid LS germline for *TLS* in 5 different restriction digests were tested for *EWS* rearrangement. One case showed a rearranged *EWS* band in *Hind*III-digested tumor DNA. RT-PCR analysis confirmed the presence of the *EWS-CHOP* fusion in this case, which is presented in more detail elsewhere.<sup>22</sup> This left one *TLS*-germline *CHOP*-rearranged case in which the putative *CHOP* translocation could not be further defined at the molecular level.



**Figure 3.** *CHOP* rearrangement analysis by Southern blotting. Rearranged bands are seen in both *Sac*I- and *Bam*HI-digested genomic DNA in four cases of classic myxoid LS (LS4, LS13, LS24, LS37) using a *CHOP* exon 3 and 4 cDNA probe (see Methods).



**Figure 4.** *TLS* rearrangement analysis by Southern blotting. Rearranged bands are seen in *Bcl*I-digested genomic DNA in two cases of classic myxoid LS (LS2 and LS15), but not in a case of predominantly myxoid WDLS (LS56) using a *TLS* exon 3–6 cDNA probe (see Methods and Figure 2).

### Predominantly Myxoid WDLs Group

Among the WDLs with >90% myxoid histology, all of the cases tested had a germline pattern with *CHOP* probe by both *Bam*HI and *Sac*I digests. In addition, 12 of 17 cases were also tested for *TLS* rearrangements and showed a germline profile: 8 by *Bcl*I digest only, 2 by both *Bcl*I and *Pst*I, and 2 by *Bam*HI digest only.

### Other WDLs and Pleomorphic LS

The other lipomatous tumors composing the control group, including LS with variable myxoid component (25 to 50%) or with absent or focal myxoid change had a germline pattern with *CHOP* probe: 14/17 cases with both *Bam*HI and *Sac*I and 3/17 cases only with *Bam*HI (technical failure with *Sac*I digest). In 9 cases, *TLS* was also studied and was not rearranged in any case. All 3 pleomorphic LS had a germline pattern for *CHOP* using both *Bam*HI and *Sac*I, and in 2/3 also with *Hind*III. Southern blot analysis with the *TLS* cDNA probe was performed in 1 of the 3 pleomorphic LS and showed a germline pattern in *Bcl*I-digested DNA.

### Myxofibrosarcomas

All myxofibrosarcomas had a germline profile with *CHOP* (12/13 cases with both *Bam*HI and *Sac*I, and 1/13 with *Bam*HI, *Eco*RI, and *Hind*III). Five cases were also tested for *TLS* and showed no genomic rearrangement.

### Discussion

The *CHOP* gene encodes a member of leucine zipper transcription factor family implicated in adipocyte differentiation and growth arrest.<sup>23,24</sup> In myxoid LS, most of the *CHOP* gene is fused to the 5' portion of *TLS* (for *translocated in liposarcoma*),<sup>6</sup> also known as *FUS* (for *fusion*).<sup>5</sup> The fusion gene encodes a protein that consists of the amino terminus of *TLS* fused to the full-length *CHOP*-coding region. The oncogenic effect of *TLS-CHOP* may be mediated at least in part by inhibition of pre-adipocyte differentiation.<sup>25-27</sup>

The *TLS* genomic breaks are clustered in introns 5 and 7<sup>18</sup> (Figure 2),<sup>19</sup> whereas the vast majority of *CHOP* breakpoints occur in intron 1 or immediately upstream of exon 1.<sup>19,20</sup> In the cases where the *CHOP* genomic break is upstream of exon 1, the latter is systematically spliced out of the mRNA, presumably because it would result in loss of the reading frame. By RT-PCR, approximately two-thirds of cases contain a hybrid transcript in which *TLS* exon 5 is fused to *CHOP* exon 2 (type II transcript), whereas in about one-third, the *TLS-CHOP* transcript also contains exons 6 and 7 of *TLS* (type I transcript).<sup>12,13,20</sup>

The extensive homology between *TLS* and *EWS* suggest that the two genes are closely related and may have originated from a common ancestor gene.<sup>28</sup> Thus, it is not entirely surprising that in rare cases of myxoid LS, the *EWS* gene at 22q12 is an alternative translocation partner of *CHOP*.<sup>7,8</sup> It has been previously difficult to estimate the

prevalence of the *EWS-CHOP* fusion in myxoid LS. In our study approximately 2% (1 of 42) of myxoid LS contain *EWS-CHOP* instead of *TLS-CHOP*. In a smaller previous study ( $n = 20$ ), *EWS-CHOP* appeared to make up 5% of myxoid LS.<sup>29</sup>

The distinction between myxoid LS and other subtypes of myxoid sarcomas is important in predicting the biological behavior and, hence, planning the management of these patients. More than other types of LS or other myxoid sarcomas of the extremities, myxoid LS are prone to metastasize to soft tissue locations, such as retroperitoneum, opposite extremity, and axilla.<sup>2,30</sup> Furthermore, myxoid LS is the predominant histological type among soft tissue sarcomas with multifocal presentation.<sup>31</sup> In a significant number of cases of myxoid LS, about 10 to 20%, patients present clinically with either synchronous or metachronous multifocal tumors.<sup>22</sup> In a recent study addressing the question of clonality by molecular analysis in six patients who presented with either synchronous or metachronous multifocal myxoid LS, we confirmed the monoclonal origin of these multifocal tumors, establishing the metastatic nature of distant soft tissue lesions in these cases.<sup>22</sup>

The characterization of the specificity and sensitivity of genomic rearrangements of *TLS* and *CHOP* genes for myxoid LS is needed to apply this test in the routine diagnostic work-up of myxoid tumors. Our results support the complete specificity and high sensitivity of *CHOP* rearrangement, due either to the *TLS-CHOP* or *EWS-CHOP* fusion, for the entity of myxoid/round cell LS. The sensitivity appears limited only by technical factors such as the quantity and quality of genomic DNA available, and the occurrence of rare cases in which detection of the rearrangement may require enzymes other than *Bam*HI and *Sac*I. In terms of specificity, the absence of *CHOP* or *TLS* gene rearrangements in 15 cases of myxofibrosarcoma in the present series is in keeping with the findings of Nilbert et al,<sup>32</sup> in which 41 cases of malignant fibrous histiocytoma (MFH), including 13 of myxoid type, had a germline pattern by Southern blot analysis of *CHOP*.

Recently, Willeke et al<sup>16</sup> reported *TLS-CHOP* fusion transcripts detected by nested RT-PCR in several cases of WDLs and pleomorphic LS. The *TLS-CHOP* fusion transcripts detected in these cases were mostly unusual in structure,<sup>16</sup> unlike the common fusions of *TLS* exon 5 or 7 to *CHOP* exon 2. These findings are in disparity with several previous RT-PCR studies<sup>9,12,13</sup> and the large cytogenetic study reported by the CHAMP group.<sup>11,14</sup> In the latter study, none of the 264 cases of various types of adipose tumors, other than myxoid LS, exhibited the t(12;16), including 19 cases of atypical lipomatous tumors with myxoid changes. The results of our study confirm the consistency of *CHOP* and *TLS* genomic rearrangements in a large group of myxoid LS by Southern blotting. The presence of these molecular alterations appears to be highly specific for the myxoid and round cell variants of LS, and none of the other histological types tested for either *CHOP* or *TLS* genes showed genomic rearrangements.

Overwhelming evidence points to the specificity of different cytogenetic changes for particular types of LS. Notably, the t(12;16)(q13.3;p11.2) is present in >95% of myxoid and round cell LS.<sup>9,14</sup> About 80% of WDLS show ring or marker chromosomes.<sup>14</sup> These are derived from 12q13–15, including amplified most typically, rearranged *HMGI-C*, and, perhaps secondarily, *MDM2*, *CDK4*, and *CHOP*. Nonetheless, occasional reports of apparent mixed-type LS (WDLS + myxoid LS) and a recent description of 3 cases of myxoid LS with areas of dedifferentiation into a high grade nonlipogenic component resembling MFH (akin to dedifferentiation in WDLS) has led to the suggestion of a closer relationship between these two types of LS than previously accepted.<sup>17</sup> We should note, however, that although dedifferentiation has been associated with a limited number of sarcoma types, it is clearly not sufficiently specific to establish biological relationships between the sarcoma types in which it has been observed. In these studies, no molecular or cytogenetic data were available.

Some cytogenetic reports have, however, seemed to contradict the clear distinction between myxoid LS and WDLS. Complex karyotypes, including ring and/or marker chromosomes, have been described in three cases of apparent mixed myxoid and well-differentiated LS.<sup>33,14</sup> In a different cytogenetic study including 31 LS samples, tumors from three of nine patients with a histological diagnosis of myxoid LS lacked the t(12;16).<sup>10</sup> In one of these patients, the tumor samples had a cytogenetic phenotype resembling that of WDLS with telomeric associations, large markers, and ring chromosomes, whereas in the other two cases, the karyotype was more akin to pleomorphic LS.<sup>10</sup> We believe that in many, if not all, of these instances of mixed myxoid + well-differentiated LS<sup>14,33</sup> or translocation-negative myxoid LS,<sup>10</sup> the tumors analyzed may have represented predominantly myxoid WDLS or pleomorphic LS with myxoid change, as supported by the cytogenetic data in the respective reports. The presence of microscopic foci of lipoma-like or sclerosing areas, characteristic of WDLS, constitutes sufficient histological evidence, in our opinion, to exclude the diagnosis of myxoid LS, as supported by our molecular analysis showing the consistent absence of *CHOP* or *TLS* genomic rearrangements in such tumors. The present results thus reinforce the concept that myxoid LS and WDLS are distinct entities and suggest that most cases that seem to contradict this sharp distinction might be due to incomplete histopathological or molecular/cytogenetic analysis. Biologically, it is of some interest to speculate on the possible basis for the preferential occurrence of the *TLS-CHOP* rearrangement in extremities, compared to retroperitoneum.

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