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Advances in Brief

Translocation t(12;16)(q13;p11) in Myxoid Liposarcoma and Round Cell Liposarcoma: Molecular and Cytogenetic Analysis[1]

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Abstract

Translocation t(12;16)(q13;p11) is regarded as a diagnostic marker for myxoid liposarcoma. Cytogenetic data on round cell liposarcomas and combined myxoid and round cell tumors is scarce, and the genetic basis of progression of myxoid tumors to high grade, round cell lesions is unknown. We have accumulated six round cell, four combined myxoid and round cell, and three myxoid liposarcomas for analysis. t(12;16)(q13;p11) was present in three round cell lesions and was detectable in all of the tumors by DNA analysis. In each tumor type, the *CHOP* gene in 12q13 was rearranged and fused to the *TLS* gene in 16p11. A variant *TLS-CHOP* RNA transcript was detected by polymerase chain reaction but did not correlate with clinicopathological data. No distinguishing cytogenetic or molecular markers for round cell or mixed lesions were found. The histogenic and genetic relatedness of myxoid and round cell liposarcomas is apparent from these data.

Introduction

Round cell liposarcoma is a relatively uncommon subtype of liposarcoma that is regarded as a poorly differentiated variant of myxoid liposarcoma. There are differences in clinical behavior, with round cell liposarcomas being highly metastatic high grade tumors, whereas myxoid liposarcomas rarely metastasize and have a more favorable 5-year survival rate (1). Diagnosis and, hence, prognostic predictions can be complicated by lesions that contain admixed components of myxoid and round cell morphology. In fact, there exists a continuous morphological spectrum between pure myxoid and pure round cell lesions, and the latter are extremely rare, since extensive sampling almost always reveals small myxoid areas. Such mixed liposarcomas (*i.e.*, combined myxoid and round cell) can recur in individual patients with an expanding round cell component and, hence, diminished prognosis. This raises interesting questions about clonal evolution, clonal expansion, and the genetic basis of phenotypic variation in liposarcomas. Three models can be considered: (*a*) that the tumors are clonal but differentiate to different extents in response to extrinsic factors; (*b*) that myxoid and round cell areas arise from unrelated progenitor cells; (*c*) that round cell clones expand as a result of accumulated gene mutations in myxoid cells.

A unique chromosome translocation t(12;16)(q13;p11) is the key genetic aberration in myxoid liposarcomas, resulting in the fusion (and transcriptional deregulation) of the *TLS* and *CHOP* genes (2). *CHOP* (also known as *Gadd153*) is a negative regulatory component of adipocyte differentiation (3, 4). Although t(12;16)(q13;p11) has been reported in mixed liposarcomas (5, 6), the cytogenetic data fails to clarify whether both cell types carry a t(12;16). In other studies, complex karyotypes without involvement of t(12;16) have been reported in round cell lesions (7, 8). In this study, we have subjected a series of round cell, mixed, and myxoid liposarcomas to cytogenetic and molecular genetic screening, the results of which provide evidence of the histogenic and genetic relatedness of these tumor types.

Materials and Methods

Tumors. A series of 13 tumors was collected at St. Thomas's Hospital, London. Extensive sampling for histological analysis was performed on each case to assess the relative proportions of myxoid and round cell areas. Lesions with a content of 5 to 80% round cell areas were classified as combined (mixed) liposarcomas. Karyotypes were determined from analysis of at least 20 G-banded metaphases.

Southern Analysis. DNA was extracted from tumors and control blood samples (9). Approximately 10 μg of each DNA was restriction endonuclease digested with 60 units *Hin*dI11 at 37°C for 5 h and size separated in a 0.75% agarose gel in 0.5X TBE (90 mM Tris-Borate, pH 8.3/2 mM EDTA) at 2.5 V/cm for 18 h. Southern blotting and hybridization of a radiolabeled *CHOP* (Gadd153) cDNA probe (3) was carried out as described (10).

RT-PCR Analyses. Tumor RNA was extracted using a guanadinium isothiocyanate method (9). One μg of total RNA was reverse transcribed using Superscript 11 reverse transcriptase (GIBCO-BRL) and poly-d(T) primer according to the supplied GIBCO-BRL protocol. The resulting cDNA[3] was amplified using *TLS-CHOP*-specific primers, 5′-CAGAGCTCCCAATCG-TCTTACGG-3′ and 5′-GAGAAAGGCAATGACTCAGCTGCC-3′, corresponding to nucleotides 331–353 and 960–983 of the *TLS-CHOP* sequence (2, 11). cDNA prepared from the lymphoblastoid cell line K562 and synovial sarcoma cell line CME-1 was included as a control. Amplification cycles were 94°C for 15 s, 62°C for 40 s, 72°C for 40 s (for 30 cycles), and final extension for 1 cycle at 72°C for 5 min.

Results

We have analyzed a case series of 13 liposarcomas from 11 patients aged between 23 and 74 years. Most of the lesions arose at sites in the limbs. In two patients, metastases developed in the retroperitoneum, axilla, and mediastinum, and these were sampled for histological and molecular evaluation. The clinical and genetic data are summarized in Table 1. Three morphological variants were recognized, and these are illustrated in Fig. 1: predominantly myxoid (sometimes with focal hypercellular round cell areas), combined (mixed) round cell and myxoid components, and pure or predominantly round cell. In six lesions, the round cell component exceeded 80%, and abnormal karyotypes were derived for three of these cases (Table 2). In each case, a chromosome translocation t(12;16)(q13;p11) was present in 20 of 20 metaphases analyzed. Noteably, case 9030 contained no detectable myxoid areas and carried a t(12;16)(q13;p11) as the sole chromosome aberration. This lesion (9030) and case 9391 were metastases of a primary tumor excised 5 years previously that showed the histological features of combined myxoid and round cell liposarcoma with an approximately 30% round cell component. This represented a good example of tumor progression to higher grade histology (no cytogenetic data was obtained for the primary tumor).

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[3] The abbreviations used are: cDNA, complementary DNA, PCR, polymerase chain reaction, bp, base pair(s).

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Table 1 *Summary of liposarcoma data*

Case no.[a]	Age/Sex	Source[b]	Phenotype[c]	t(12;16)(q13;p11)	Bands detected[d] with *CHOP* probe	*TLS-CHOP* junction[e]
[10948	25/M	1°	Round (90%)	–	10/6.5	+
L10473		2°	Round (80%)	+	10	ND
[9030	29/M	2°	Round (100%)	+	10/6.7	+
L9391		2°	Round (95%)	+	10/6.	ND
777	69/M	1°	Round (80%)	ND	9.7/9.2	+
7914	63/M	1°	Round (90%)	ND	ND	+
13583	48/M	1°	Mixed (50%)	ND	9.2/7.0	+
12144	48/M	1°	Mixed (50%)	+	ND	ND
356	74/M	1°	Mixed (15%)	ND	ND	+
10776	37/M	1°	Mixed (5%)	ND	9.7	+
7569	26/M	1°	Myxoid	ND	9.7/>12	+
2159	54/M	1°	Myxoid	ND	ND	+
9275	23/F	1°	Myxoid	ND	ND	+

[a] Samples from same patient are bracketed.
[b] 1°, primary; 2°, metastasis.
[c] Proportion of round cells is expressed as a percentage of sectioned area.
[d] Based on Southern analysis of *Hind*III-cleaved DNA. DNA fragment sizes are estimated in kilobases.
[e] PCR assay (see also Fig. 2); ND, not determined.

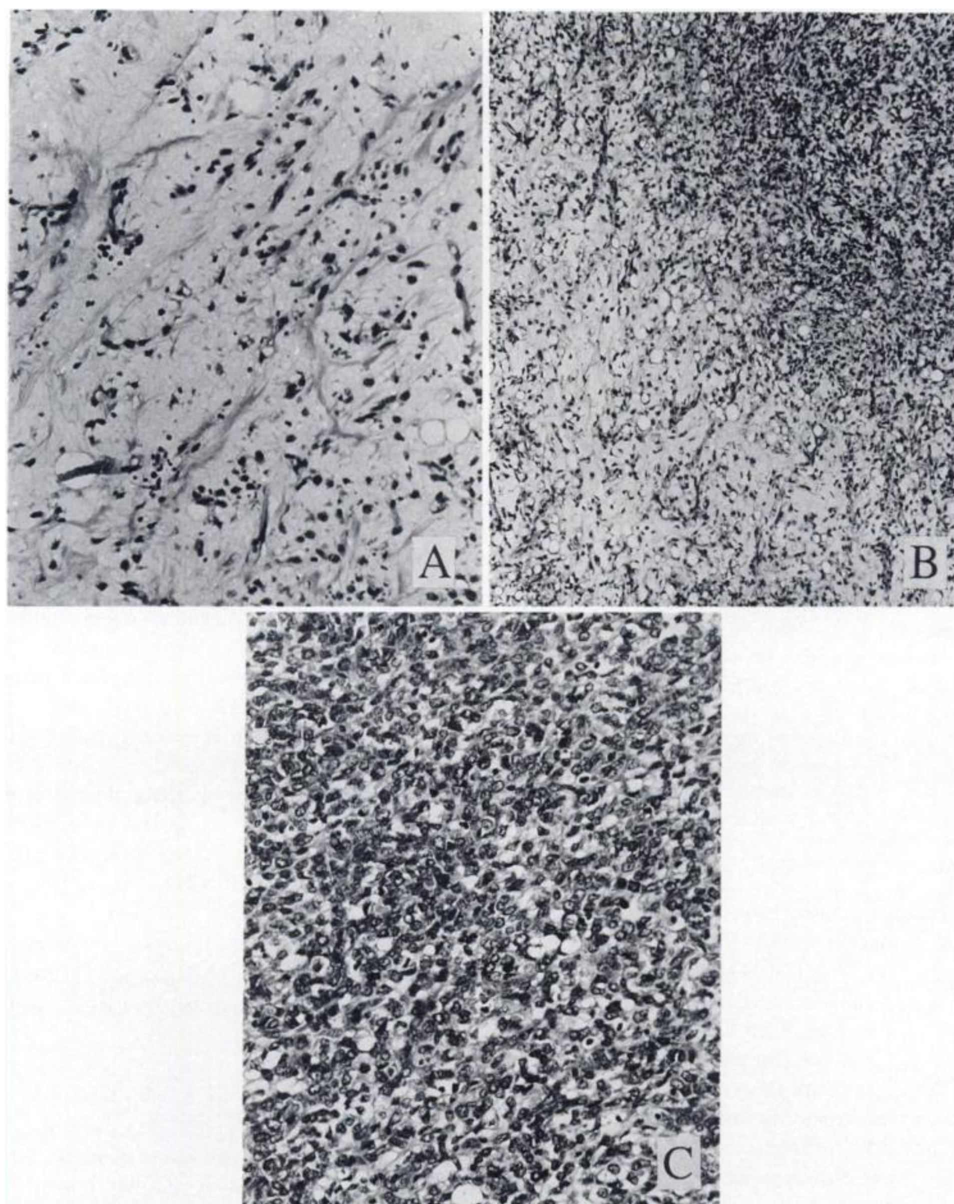

Fig. 1. A spectrum of morphological types in liposarcomas characterized by t(12;16)(q13;p11). *A*, typically hypocellular myxoid liposarcoma. *B*, mixed (combined myxoid and round cell) liposarcoma. *C*, round cell liposarcoma (note the scattered lipoblasts).

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Table 2 *Karyotypes of liposarcomas*

Case[a]	Source[b]	Karyotype
[9030	2°	46,XY,t(12;16)(q13;p11)
9391]	2°	Near tetraploid,t(12;16)(q13;p11), + other chromosomal changes
[10948	1°	46,XY
10473]	1°	46,XY,t(12;16)(q13;p11),6q−
12144	1°	46,XY,t(12;16)(q13;p11)

[a] Tumors from same patient are bracketed.
[b] 1°, primary; 2°, metastasis.

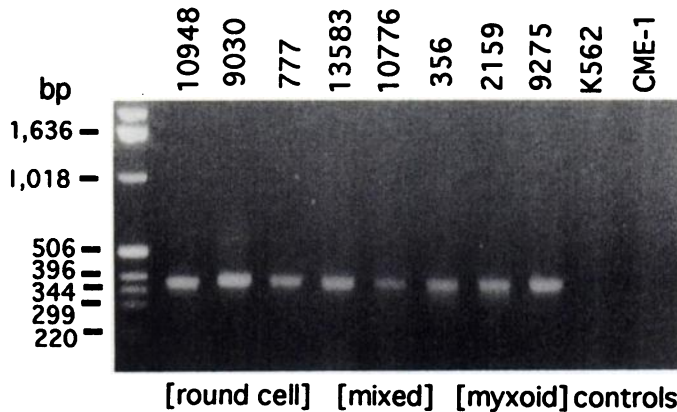

Fig. 2. Detection of *TLS-CHOP* transcripts by RT-PCR. All tumors screened (see Table 1) generated a product of approximately 370 bp, corresponding to the Type 2 junction sequence described recently (11). Examples of round cell, myxoid, and mixed liposarcomas are shown. DNA primers and PCR conditions are specified in "Materials and Methods." The migration distances of kilobase ladder markers (GIBCO-BRL) are indicated.

Southern analysis with a *CHOP* (*Gadd153*) cDNA probe revealed abnormal hybridizing bands compared to constitutional DNA from the same patients (data summarized in Table 1). A single *Hin*dIII-cleaved DNA fragment of 10-kb was observed in blood lymphocyte samples. In 3 of 8 liposarcomas, one wild-type and one aberrant band were detected. In an additional 3 of 8 cases, two aberrant bands and no wild-type band were observed. In one case (10776), a single aberrant band and no wild-type band was observed. No rearrangement of *CHOP* was detected in a metastasis (10473) of tumor 10948, although a t(12;16) translocation was present in cells from 10473, and the primary tumor carried a *CHOP* gene rearrangement. The negative result was probably due to nonrepresentative sampling. There was no evidence of *CHOP* gene amplification in any of the tumors.

PCR amplification of cDNA prepared from tumor RNA showed that a hybrid *TLS-CHOP* sequence (2) was present in all liposarcomas tested (Fig. 2). The PCR product was unique to liposarcomas and was not present in control cell lines derived from other tissues. Based on the published sequence of the *TLS-CHOP* junction (2), the DNA primers used for PCR analysis were predicted to generate a product of 654 bp. However, we saw a smaller than expected product of approximately 370 bp in all liposarcomas tested (Fig. 2).

Discussion

The recurrent chromosome translocation t(12;16)(q13;p11) has been widely reported as a specific marker for myxoid liposarcoma (12). Our studies show that t(12;16)(q13;p11) is also present in round cell liposarcomas and mixed liposarcomas (combined myxoid and round cell). Cytogenetic data on round cell tumors has been scarce. Three lesions with admixed round cell and myxoid areas were found to contain cells with t(12;16)(q13;p11) as the sole chromosome change (5, 6), but it is not clear from this data whether cells of both phenotypes carried the chromosome aberration, and the relative proportions of round cell and myxoid components were not documented. Karyotyping of an extremely rare case of pure (100%) round cell tumor has eliminated any possible sampling bias in our results. t(12;16)(q13;p11) was present in this and two other lesions histologically shown to have round cell areas accounting for between 80 and 95% of the surface area examined. Molecular analyses subsequently confirmed the presence of t(12;16)(q13;p11) in three additional round cell liposarcomas. Reports of only three other cases are documented, and these had complex karyotypes without visible involvement of bands 12q13 and 16p11 (7, 8). These investigators concluded that round cell and myxoid lesions were not related cytogenetically. Our results contradict this conclusion.

Molecular dissection of the myxoid liposarcoma t(12;16)(q13;p11) translocation has shown that two genes, *TLS* and *CHOP*, are fused and transcribed as an aberrant chimeric RNA transcript (2). We have demonstrated that *CHOP* gene sequences on chromosome 12 are rearranged also in round cell and mixed liposarcomas and that the *TLS-CHOP* fusion transcript is present in these tumors. Similar to the Southern data of Aman *et al.* (13), we noted several abnormal DNA restriction fragment patterns resulting from the t(12;16) rearrangement in different tumors, but these patterns did not correlate with tumor type. No detectable amplification of *CHOP* sequences had occurred in these liposarcomas.

Specific analysis of a region across the *TLS-CHOP* junction showed that the fusion point between the two genes is invariant between myxoid liposarcoma, combined myxoid and round cell liposarcoma, and pure round cell liposarcoma. Panagopoulos *et al.* (11) have described variant fusion points in *TLS* in 12 liposarcomas. These were detected as 654-bp (Type 1) and 370-bp (Type 2) bands by RT-PCR across the *TLS-CHOP* junction. Some tumors contained both variants of the *TLS-CHOP* cDNA. Sequencing of the smaller band (actual length, 378 bp) showed that the break was located 275 bp upstream of the *TLS* breakpoint described in the original characterization of t(12;16; Ref. 2). Using a similar approach and identical primers for DNA amplification, we have identified an additional 13 tumors with this Type 2 variant 378-bp chimeric cDNA. None of the cases we examined contained the 654-bp cDNA. Together with the data of Panagopoulos *et al.* (11), it appears that the Type 2 variant results more frequently from the t(12;16) translocation than the Type 1 variant. We do not observe any correlation between the upstream fusion point in *TLS* and tumor phenotype or any other clinicopathological parameter.

In conclusion, myxoid and round cell liposarcomas share a key genetic defect, the fusion of truncated *TLS* and *CHOP* genes. The mechanism by which this chimeric gene induces cell transformation and determines tumor phenotype is unknown. Our results suggest the potential for both morphological variants to expand from the same progenitor cell with t(12;16)(q13;p11). We find no evidence that tumor progression from myxoid to round cell morphology results from *CHOP* gene amplification or secondary chromosome aberrations. We suggest that the plasticity of phenotype between myxoid and round cell liposarcoma is induced by extrinsic factors or by gene mutations undetected by cytogenetic analysis and unrelated to the translocation t(12;16)(q13;p11).

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References

1. Enzinger, F. M., and Weiss, S. W. (eds.). Soft Tissue Tumours, Ed. 2, pp. 246–382. St. Louis, MO: The Mosby Company, 1988.
2. Crozat, A., Aman, P., Mandahl, N., and Ron, D. Fusion of *CHOP* to a novel RNA-binding protein in human myxoid liposarcoma. Nature (Lond.), *363:* 640–644, 1993.
3. Park, J. S., Luethy, J. D., Wang, M. G., Fargnoli, J., Fornace, A. J., Jr., McBride, O. W., and Holbrook, N. J. Isolation, characterization and chromosomal localization of the human *GADD153* gene. Gene (Amst.), *116:* 259–267, 1992.
4. Ron, D., and Habener, J. F. CHOP, a novel developmentally regulated nuclear protein

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that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. Genes Dev., *6:* 439–453, 1992.
5. Mertens, F., Johansson, B., Mandahl, N., Heim, S., Bennet, K., Rydholm, A., Willen, H., and Mitelman, F. Clonal chromosome abnormalities in two liposarcomas. Cancer Genet. Cytogenet., *28:* 137–144, 1987.
6. Eneroth, M., Mandahl, N., Heim, S., Willen, H., Rydholm, A., Alberts, K. A., and Mitelman, F. Localization of the chromosomal breakpoints of the t(12;16) in liposarcoma to subbands 12q13.3 and 16p11.2. Cancer Genet. Cytogenet., *48:* 101–107, 1990.
7. Molenaar, W. M., DeJong, B., Buist, J., Idenburg, V. J. S., Seruca, R., Vos, A. M., and Hoekstra, H. J. Chromosomal analysis and the classification of soft tissue sarcomas. Lab. Invest., *60:* 266–274, 1989.
8. Ohjimi, Y., Iwasaki, H., Kaneko, Y., Ishiguro, M., Ohgami, A., Fujita, C., Shinohara, N., Yoshitake, K., and Kikuchi, M. Chromosome abnormalities in liposarcomas. Cancer Genet. Cytogenet., *64:* 111–117, 1992.
9. Maniatis, T., Fritsch, E. F., and Sambrook, J. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
10. Knight, J. C., Reeves, B. R., Kearney, L., Monaco, A. P., Lehrach, H., and Cooper, C. S. Localization of the synovial sarcoma t(X;18)(p11.2;q11.2) breakpoint by fluorescence *in situ* hybridization. Hum. Mol. Genet., *1:* 633–637, 1992.
11. Panagopoulos, I., Mandahl, N., Ron, D., Hoglund, M., Nilbert, M., Mitelman, F., and Aman, P. Characterization of the *CHOP* breakpoints and fusion transcripts in myxoid liposarcomas with the 12;16 translocation. Cancer Res., 6500–6503, 1994.
12. Sandberg, A. A., and Bridge, J. A. (eds.). The Cytogenetics of Bone and Soft Tissue Tumors. Austin, TX: R. G. Landes Company, 1994.
13. Aman, P., Ron, D., Mandahl, N., Fioretos, T., Heim, S., Arheden, K., Willen, H., Rydholm, A., and Mitelman, F. Rearrangement of the transcription factor gene *CHOP* in myxoid liposarcomas with t(12;16)(q13;p11). Genes Chromosomes Cancer, *5:* 278–285, 1992.

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