American Journal of Pathology, Vol. 147, No. 5, November 1995

Short Communication

Chimeric *TLS/FUS-CHOP* Gene Expression and the Heterogeneity of its Junction in Human Myxoid and Round Cell Liposarcoma

Masahiko Kuroda,*† Tsuyoshi Ishida,† Hajime Horiuchi,† Naotoshi Kida,† Hiroshi Uozaki,*† Hajime Takeuchi,* Kaori Tsuji,‡ Tetsuo Imamura,‡ Shigeo Mori,* Rikuo Machinami,† and Toshiki Watanabe*

From the Department of Pathology, * *The Institute of Medical Science and Department of Pathology, Faculty of Medicine,*† *The University of Tokyo, Tokyo, Japan; and Department of Surgical Pathology,*‡ *Faculty of Medicine, Teikyo University, Tokyo, Japan*

Myxoid liposarcomas have a unique and specific t(12;16)(q13;p11) chromosomal translocation. The breakpoint has recently been identified and shown to involve the* TLS/FUS *gene on chromosome 16 and the* CHOP *gene on chromosome 12. This translocation causes fusion of these genes resulting in the expression of a novel chimeric* TLS/FUS-CHOP *message. Using the polymerase chain reaction with primer sets derived from sequences of* TLS/FUS *and* CHOP *cDNAs, we could amplify three types of the fusion transcripts from seven of seven samples of myxoid and round cell liposarcomas. In six of the seven positive samples, two kinds of chimeric messenger RNAs were found that have been reported previously. However, the last sample had a novel chimeric message that had an extra sequence of 33 bp derived from the* TLS/FUS *gene. Thus, it was shown that these fusion transcripts had a varying extent of the sequence of* TLS/FUS *gene incorporated at the site of the fusion. However, the* TLS/FUS-CHOP *fusion transcripts were not detected in two pleomorphic liposarcomas or in three myxoid variants of malignant fibrous histiocytomas. Our findings indicate that in liposarcomas* TLS/FUS-CHOP *fusion transcripts have variations at the junction of chimeric messages, which was the case for Ewing's sarcoma. Detection of the chimeric message by reverse transcription polymerase chain reaction was also suggested to be a useful approach for the diagnosis of myxoid and round cell liposarcomas that have (12;16) translocation, and for distinguishing them from pleomorphic liposarcoma and myxoid variant of malignant fibrous histiocytomas. (Am J Pathol 1995, 147:1221–1227)

Liposarcoma is one of the most common soft tissue sarcomas of adulthood. Liposarcoma originates in primitive mesenchymal cells rather than mature adipose tissue.[1] According to the classification of Enzinger and Weiss,[1] liposarcomas are classified into four basic histological categories: 1) myxoid liposarcoma, 2) round cell liposarcoma, 3) well differentiated liposarcoma, and 4) pleomorphic liposarcoma, *which corresponds well to those of the World Health Organization (WHO) classification.* Myxoid liposarcoma is the most common variant and accounts for up to 50% of all liposarcomas. Histologically, the tumor is composed of lipoblasts in various stages of differentiation with a prominent plexiform capillary pattern and abundant myxoid matrix. Although round cell liposarcoma is closely related to myxoid liposarcoma and seems to represent its poorly differentiated form,[2] it deserves separate consideration be-

Supported in part by grants-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan to MK, RM, and TW; and by the Smoking Research Foundation to RM.

Accepted for publication July 17, 1995.

Address reprint requests to Toshiki Watanabe, Department of Pathology, The Institute of Medical Science, The University of Tokyo, 4–6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan.

cause of its aggressive clinical course and metastatic potential.[2]

Myxoid liposarcomas share a specific reciprocal translocation between band q13 on chromosome 12 and band p11 on chromosome 16.[3–8] The chromosomal breakpoints involved in t(12;16) have recently been identified at a molecular level.[9,10] The breakpoint on chromosome 12 involves the transcription factor *CHOP* gene, a member of the CCAAT/enhancer binding protein family, which may be involved in adipocyte differentiation.[11,12] The CHOP protein has a leucine zipper dimerization domain, and it can bind to CATA-box-binding (C/EBP-like) proteins, blocking their interaction with DNA.[11,12] The *CHOP* gene is rearranged with a gene on chromosome 16, designated *TLS* or *FUS*.[9,10] The *TLS/FUS* has an extensive similarity to the recently described *EWS* gene,[13] which has been shown to be rearranged in Ewing's sarcoma/primitive neuroectodermal tumors[13] and clear cell sarcoma.[14] Like the EWS protein, the TLS/FUS protein has a glutamine-serine-tyrosine-rich segment, three glycine-rich stretches,[9,10] and an RNA-binding domain.[9]

The structural consequences of these translocations were revealed on liposarcoma,[9,10] Ewing's sarcoma,[13] rhabdomyosarcoma,[15] and synovial sarcoma.[16] One of the characteristics of these chromosomal translocations is that they all have the genes for the DNA-binding factors, resulting in expression of fusion transcripts. The function of these chimeric proteins and their roles in tumor genesis have not been well clarified. Sanchez-Garcia and Rabbitts[17] showed that the N-terminal domain of the TLS/FUS-CHOP protein can act as a transcriptional transactivator. Barone et al[18] have reported that the TLS/FUS-CHOP protein blocks the induction of G1/S arrest by native CHOP protein, suggesting a role of the chimeric protein in the establishment of myxoid liposarcoma.

Two types of translocations, *EWS/Fli1* and *EWS/ERG*, have been reported in Ewing's sarcoma/primitive neuroectodermal tumors, and the fusion transcripts have been revealed to have considerable variations in the sequences around the junction.[13,19–23] As for liposarcomas, Panagopoulos et al[24] recently found a variant (type II) of the chimeric *TLS/FUS-CHOP* transcript,[24] suggesting that the junction of the chimeric *TLS/FUS-CHOP* mRNAs also shows considerable variation. However, the possible functional diversity among the variant fusion proteins remains to be studied.

In this study, we examined the expression of the *TLS/FUS-CHOP* fusion transcripts by means of reverse transcription polymerase chain reaction (RT-PCR) in malignant myxoid tumors including myxoid liposarcoma, round cell liposarcoma, pleomorphic liposarcoma, and a myxoid variant of malignant fibrous histiocytoma (MFH). We also studied the relationship between the histological subclassification of liposarcoma and the types of *TLS/FUS-CHOP* fusion transcripts.

Materials and Methods

Tumors

The tumors used in this study were as follows: four myxoid liposarcomas (Figure 1A), two mixed type liposarcomas (myxoid with round cell areas, Figure 1B), one round cell liposarcoma (Figure 1C), two pleomorphic liposarcoma, and three tumors of myxoid variant of MFH. All these tumors were diagnosed by detailed histopathological observation. Among these, the tumor of case 8 showed unusual features corresponding to those of round/pleomorphic liposarcoma in Evans'[25] classification. However, we diagnosed it as pleomorphic liposarcoma according to the WHO classification.

PCR Analysis

Total RNA was extracted using RNA zolB (Biotecx, Houston, TX). Template cDNA was synthesized from 0.5 μg of total RNA using Superscript (Life Technologies, Inc., Gaithersburg, MD). One-tenth of the cDNA was used as template DNA for PCR amplification. Amplified sequences were resolved by electrophoresis on a 1% agarose gel, and were extracted from the gel with Geneclean II (BIO 101 Inc., Vista, CA) followed by subcloning into the pCRII vector of the TA cloning kit (Invitrogen, San Diego, CA). The nucleotide sequences of the primers are as follows: 5′A; 5′-GGCAATCAAGACCAGAGTGG-3′ (nucleotide position 661–680), 5′B; 5′-TTATAGCCAGTCCACGGACA-3′ (nucleotide position 198–217), 3′A; 5′-TCATACCAGGCTTCCAGCTC-3′ (nucleotide position 1006–1025). PCR proceeded for 40 cycles in a 50 μl reaction mixture containing 200 μmol/L of each of four deoxynucleotide triphosphates, 0.1 μmol/L of each primer and 1 unit of Taq polymerase (Boehringer Mannheim, Germany).

Genomic DNA Analysis

DNA was extracted from the frozen specimens and when available from peripheral blood lymphocytes using standard methods.[28] For Southern blot analysis of the rearranged *CHOP* gene, 10 μg of *Sst*I-

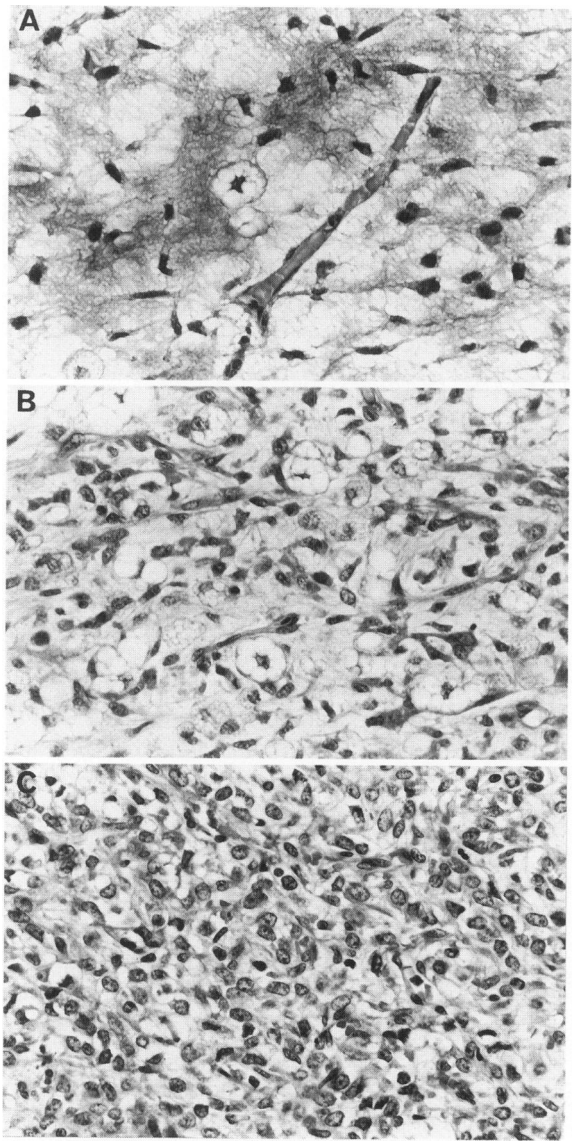

Figure 1. A: *Myxoid liposarcoma (case 1, type I). Proliferation of lipoblasts, with plexiform capillary network and abundance of myxoid material. (H&E, ×456).* **B:** *Mixed type (myxoid and round cell liposarcoma) (case 4, type III). Transitional area between myxoid and round cell liposarcoma consisting of numerous undifferentiated tumor cells in addition to scattered multivacuolated lipoblasts. (H&E, ×456).* **C:** *Round cell liposarcoma (case 7, type II) showing uniform, round cell proliferation. The vascular pattern is obscured by cellular proliferation, and there is a scattering of lipoblasts. (H&E, ×456).*

digested genomic DNA was used for agarose gel electrophoresis followed by capillary transfer with 20X SSC to positively charged nylon membranes (Biodine B, East Hills, NY). After ultraviolet fixation, hybridization was performed in a buffer containing 0.2 mol/L $NaHPO_4$, 1 mmol/L EDTA, 1% bovine serum albumin, and 7% sodium dodecyl sulfate (SDS) at 60°C for 12 hours with IN2 probe[24,27] that was ^{32}P-labeled by a random prime labeling kit (Megaprime, Amersham International plc, Little Chalfont, UK). The membranes were washed in a buffer containing 2X SSC and 0.1% SDS at room temperature for 30 minutes and then in 0.5X SSC and 0.1% SDS at 65°C for 30 minutes. Detection of the hybridization signals was performed by the use of image analyzer (BAS2000 system, Fuji Film, Tokyo, Japan).

DNA Sequence Analysis

The nucleotide sequence of the insert DNA was determined using a T7 Sequencing Kit (Pharmacia LKB Biotech Inc, Uppsala, Sweden). More than two plasmid clones for each PCR product were determined to discriminate *in vivo* mutations from base substitutions caused by misincorporation by Taq polymerase.

Results

Detection of the Chimeric mRNAs

The initial RT-PCR reaction was performed using the primer set of 5′A and 3′A that are located in the glycine-rich segment of *TLS/FUS* and exon 3 of *CHOP*, respectively (Figure 2C).[9,10] The chimeric mRNA was amplified in four of the seven samples of myxoid, mixed, and round cell liposarcomas. One of the amplified products, had the same size as the originally described chimeric mRNA (type I), and the other was larger than the size of type I transcript (Figure 2B). PCR analysis using another pair of primers, 5′B and 3′A, detected the chimeric transcript in all three samples that were negative in the initial primer pair (Table 1). Together, all seven samples studied expressed the *TLS/FUS-CHOP* chimeric transcripts: four of myxoid liposarcomas, two of mixed type liposarcomas, and one round cell liposarcoma. The chimeric messages were not detected in two pleomorphic liposarcomas, and in all the three samples of myxoid variant of MFH.

Nucleotide Sequence Analysis of the Junction of the Chimeric Messages

Analysis of the nucleotide sequences of the amplified cDNA revealed that the 364 bp sequence amplified by 5′A–3′A primer pair was identical to the originally described *TLS/FUS-CHOP* chimeric transcript (type I),[9,10] and the other 397 bp fragment was a novel chimeric transcript having an extra 33 bp at the junction in addition to the type I transcript. This extra sequence was derived from the 3′ terminus in

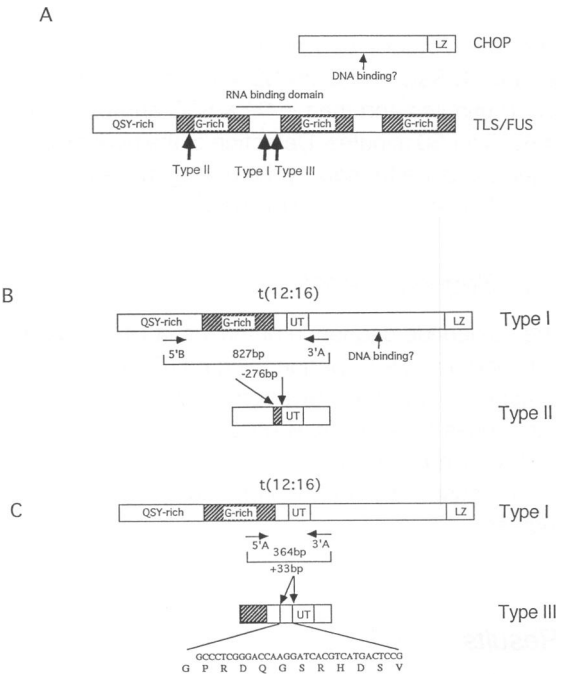

Figure 2. A: *Schematic diagram of the* TLS/FUS *and* CHOP *genes and their predicted protein products.*[20] *The t(12;16) involves the* CHOP *gene on chromosome 12 and a gene designated* TLS/FUS *on chromosome 16. The large black inverted arrows indicate translocation fusion points. The* CHOP *gene encodes a leucine zipper protein. TLS/FUS is a glycine-rich protein (cross-hatched region) together with a glutamine-serine-tyrosine (QSY)-rich N-terminal portion and a domain homologous to RNA-binding protein.* **B** *and* **C:** *Diagram of the t(12;16)-specific* TLS/FUS-CHOP *fusion transcripts (types I to III) and the location of oligonucleotide primers for the RT-PCR analysis. The breakage in types I and III in* TLS/FUS *excludes the RNA-binding domain from the* TLS/FUS-CHOP *fusion but includes the QSY-rich stretch and one of the glycine-rich stretches. However, type II further excludes the glycine-rich stretch.*[24] *The breakage in* CHOP *leaves the coding sequences intact and includes a segment translated from a normally noncoding exon from* CHOP. **C:** *Additional 33 base pairs, nucleotide sequence of the type III complementary DNA* TLS/FUS-CHOP *fusion transcript obtained by RT-PCR and the predicted amino acid sequence.*

TLS/FUS, and combined with the entire exon 2 of *CHOP*, which results in the addition of 11 amino acids to the fusion protein (Figure 2C). We designated this new chimeric transcript as type III.

Using the 5′B–3′A primer pair, we detected a 551 bp fragment that corresponded to the size of the type II chimeric transcript. The nucleotide sequence analysis confirmed that this cDNA fragment actually represented the type II transcript of *TLS/FUS-CHOP* (Figure 2B).[24]

Genomic Southern Blot Analysis

The genomic rearrangement of *CHOP* gene was studied by Southern blotting with IN2 probe that can detect rearranged *CHOP* gene[24] using *Sst*I-digested genomic DNA from four samples of liposarcoma (one myxoid, one round cell, and two pleomorphic liposarcomas). The results demonstrated a rearranged band of *CHOP* gene in addition to that of normal constitutional configuration in myxoid and round cell liposarcoma (cases 1 and 7), but not in two pleomorphic liposarcomas (cases 8 and 9) (Table 1 and Figure 3B), which is in agreement with the previous reports of Southern blot analysis[24] and classic karyotyping studies.[4,8]

Discussion

Using RT-PCR, we have demonstrated the presence of *TLS/CHOP* chimeric transcripts in all samples with the histological diagnosis of myxoid and round cell liposarcomas. Southern blot studies to detect the rearranged *CHOP* gene provided evidence verifying that results of our RT-PCR study reflected the presence or absence of its rearrangement. In terms of the classification of liposarcomas, Ohjimi et al[7] have pro-

Table 1. *Summary of Clinicopathological Data and Charactor of Fusion Products of Myxoid Tumors*

Case	Age/Sex	Site	Histological diagnosis	Chimeric message	Type of fusion product	Bands detected with IN2 probe*
1	40/F	Buttock	Myxoid LPS	+	I	N, R (6.4)
2	56/F	Leg	Myxoid LPS	+	I	nd
3	36/F	Flank	Myxoid LPS	+	I	nd
4	43/F	Neck	Myxoid/round LPS	+	III	nd
5	32/M	Thigh	Myxoid/round LPS	+	II	nd
6	27/F	Thigh	Myxoid LPS	+	II	nd
7	33/M	Leg	Round cell LPS	+	II	N, R (3.4)
8	59/F	Retro	Pleomorphic LPS	–		N
9	55/M	Thigh	Pleomorphic LPS	–		N
10	78/F	Retro	Myxoid MFH	–		nd
11	66/M	Thigh	Myxoid MFH	–		nd
12	57/M	Buttock	Myxoid MFH	–		nd

Retro: Retroperitoneum, LPS: Liposarcoma.
*Based on Southern analysis of *Sst*I-cleaved DNA. Rearranged DNA fragment sizes are estimated in kilobases. N, normal, constitutional fragment; R, abnormal rearranged fragment; nd, not determined.

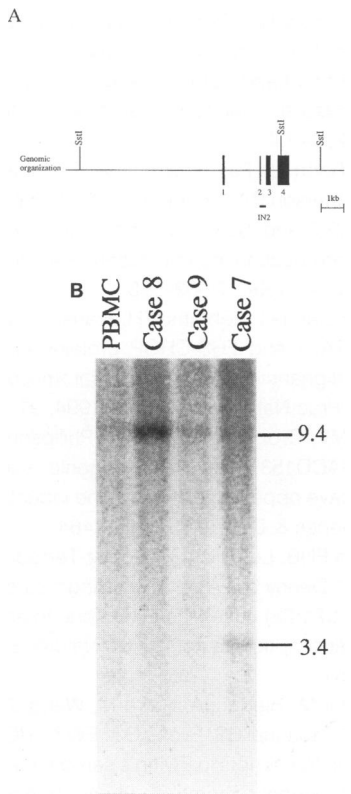

Figure 3. A: *Restriction map of* CHOP *gene. The position and length of the IN2 probe are indicated.*[24] B: *Southern blot analysis of SstI digested DNA from myxoid, round, and pleomorphic liposarcoma and normal peripheral blood mononuclear cells. The case numbers are indicated at the top of each lane. PBMC, normal peripheral blood mononuclear cells. The size of the hybridized bands are indicated at the right of the figure in kb.*

posed three subgroups on the basis of their detailed cytological analysis. The first group is characterized by the presence of t(12;16)(q13;p11), the second group has a tendency to occur in the retroperitoneum,[8] chromosomal abnormalities of ring chromosomes, telomeric associations and giant markers, and the last by complex numerical and structural aberrations.

Very recently, Knight et al[28] found the chimeric transcript of the *TLS/FUS-CHOP* gene in round cell liposarcoma, suggesting that myxoid and round cell liposarcomas have common chromosomal aberrations. The present findings are consistent with their observations and provide another support for discriminating myxoid and round cell liposarcomas from other malignant myxoid tumors including pleomorphic liposarcoma and a myxoid variant of MFH. We could not detect the fusion transcripts or genomic rearrangement of *CHOP* gene in two pleomorphic liposarcomas, one of which arose in retoperitoneum and showed unusual histological features. This could be due to the different breakpoints of the translocation, or a lack of the specific chromosomal translocations, or the presence of alternative combinations of chromosomal translocations that have not been recognized in these liposarcomas. Although our present study did not provide clues to discriminate these possibilities, absence of typical rearrangements in the *CHOP* gene suggested that the second group of the classification of Ohjimi et al may constitute a genetically distinct entity with unique histological and clinical characteristics.

We described the third type of the chimeric transcript (type III) in liposarcoma. Thus our study demonstrated that at least three types of the chimeric transcripts are expressed in liposarcomas, suggesting the presence of variation in the junction of the *TLS/FUS-CHOP* chimeric transcripts as was the case for the *EWS-Fli1* of Ewing's sarcoma. Detection and characterization of the variant chimeric mRNA will help us to understand the underlying mechanism for chimera formation, and the biological function and active domains of the protein.

Panagopoulos et al[24] reported that the type II fusion transcript is most commonly expressed in myxoid liposarcoma. On the other hand, Knight et al[28] detected only the type II fusion transcripts in all 10 samples of myxoid and round cell liposarcomas.[28] Our findings demonstrated that the type II transcript is expressed in one myxoid liposarcoma, one mixed type liposarcoma, and one round cell liposarcoma. Taken together, these findings suggest that the round cell liposarcoma expresses only the type II transcript. This notion should be confirmed by a survey on a larger number of samples, to determine whether the type II transcripts play an important role in the specific histological subtype of liposarcomas, which have a poorer prognosis.

Our findings also indicated that the presence of the chimeric *TLS/FUS-CHOP* transcripts can be pathognomonic for the most liposarcomas, and help to distinguish them from pleomophic liposarcoma and myxoid MFH, which is sometimes a diagnostic challenge to surgical pathologists. The diagnostic value of the chimeric transcripts should be clarified by an extended study on a larger number of patients.

Acknowledgments

We would like to express thanks to Dr. Mitsuru Hara, Department of Pathology, Toranomon Hospital, Tokyo, for providing surgical materials.

References

1. Enzinger FM, Weiss SW: Soft Tissue Tumors, ed 2. St. Louis, CV Mosby, 1988, 346–382
2. Weiss SW: World Health Organization International Histological Classification of Tumors, Histological Typing of Soft Tissue Tumors, ed 2. Berlin, Springer-Verlag, 1994, 25–26
3. Turc-Carel C, Limon J, Dal Cin P, Rao U, Karakousis C, Sandberg AA: Cytogenetic studies of adipose tissue tumors. II. Recurrent reciprocal translocation t(12; 16)(q13;p11) in myxoid liposarcomas. Cancer Genet Cytogenet 1986, 23:291–299
4. Sreekantaiah S, Karakousis CP, Leong SPL, Sandberg AA: Cytogenetic findings in liposarcoma correlate with histopathologic subtypes. Cancer 1992, 69:2484–2495
5. Sreekantaiah S, Ladanyi M, Rodriguez E, Chaganti RSK: Chromosomal aberrations in soft tissue tumors. Am J Pathol 1994, 144:1121–1134
6. Mrózek K, Karakousis CP, Bloomfield CD: Chromosome 12 breakpoints are cytogenetically different in benign and malignant lipogenic tumors: localization of breakpoints in lipoma to 12q15 and myxoid liposarcoma to 12q13.3. Cancer Res 1993, 53:1670–1675
7. Ohjimi Y, Iwasaki H, Kaneko Y, Ishiguro M, Ohgami A, Fujita C, Shinohara N, Yoshitake K, Kikuchi M: Chromosome abnormalities in liposarcomas. Cancer Genet Cytogenet 1992, 64:111–117
8. Karakousis CP, Cin PD, Turc-Carel C, Limon J, Sandberg AA: Chromosomal change in soft-tissue sarcoma. Arch Surg 1987, 122:1257–1260
9. Crozat A, Åman P, Mandahl N, Ron D: Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 1993, 363:640–644
10. Rabbitts TH, Forester A, Larson R, Nathan P: Fusion of the dominant negative transcription regulator *CHOP* with a novel gene *FUS* by translocation t(12;16) in malignant liposarcoma. Nature Genet 1993, 4:175–180
11. Ron D, Habener JF: CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant negative inhibitor of gene transcription. Genes & Dev 1992, 6:439–453
12. Fornace AJ Jr, Nebert DW, Hollander MC, Luethy JD, Papathanasiou M, Fargnoli J, Holbrook NJ: Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. Mol Cell Biol 1989, 9:4196–4203
13. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, Kovar H, Joubert I, De Jong P, Rouleau G, Aurias A, Thomas G: Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumors. Nature 1992, 359:162–165
14. Zucman J, Delattre O, Desmaze C, Epstein AL, Stenman G, Speleman F, Fletcher CDM, Aurias A, Thomas G: EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. Nature Genet 1993, 4:341–345
15. Galili N, Davis RJ, Fredericks WJ, Mukhopadhyay S, Rauscher III FJ, Emanuel BS, Rovera G, Barr FG: Fusion of a fork head domain gene to PAX3 in the solid tumor alveolar rhabdomyosarcoma. Nature Genet 1993, 5:230–235
16. Clark J, Rocques PJ, Crew AJ, Gill S, Shipley J, Chan AML, Gusterson BA, Cooper CS: Identification of novel genes, SYT and SSX, involved in the t(X;18)(p11.2; q11.2) translocation found in human synovial sarcoma. Nature Genet 1994, 7:502–508
17. Sanchez-Garcia I, Rabbitts, TH: Transcriptional activation by TAL1 and FUS-CHOP proteins expressed in acute malignancies as a result of chromosomal abnormalities. Proc Natl Acad Sci USA 1994, 91:7869–7873
18. Barone MV, Crozat A, Tabaee A, Philipson L, Ron D: CHOP(GADD153) and its oncogenic variant, TLS-CHOP, have opposing effects on the induction of G1/S arrest. Genes & Dev 1994, 8:453–464
19. Sorensen PHB, Lessnick SL, Lopez-Terrada D, Liu XF, Triche TJ, Denny CT: A second Ewing's sarcoma translocation, t(21;22), fuses the *EWS* gene to another ETS-family transcription factor, ERG. Nature Genet 1994, 6:146–151
20. Giovannini M, Biegel JA, Serra M, Wang JY, Wei YH, Nycum L, Emanuel BS, Evans GA: EWS-ERG and EWS-Fli 1 fusion transcripts in Ewing's sarcoma and primitive neuroectodermal tumors with variant translocations. J Clin Invest 1994, 94:489–496
21. Downing JR, Head DR, Parham DM, Douglass EC, Hulshof MG, Link MP, Motroni TA, Grier HE, Curcio-Brint AM, Shapiro DN: Detection of the (11;22)(q24; q12) translocation of Ewing's sarcoma and peripheral neuroectodermal tumor by reverse transcription polymerase chain reaction. Am J Pathol 1993, 143:1294–1300
22. Zucman J, Melot T, Desmaze C, Ghysdael J, Plougastel B, Peter M, Zucker JM, Triche TJ, Sheer D, Turc-Carel C, Ambros P, Combaret V, Lenoir G, Aurias, Thomas G, Delattre O: Combinatorial generation of variable fusion proteins in the Ewing family of tumors. EMBO J 1993, 12:4481–4487
23. Delattre O, Zucman J, Melot T, Garau XS, Zucker JM, Lenoir GM, Ambros PF, Sheer D, Turc-Carel C, Triche TJ, Aurias A, Thomas G: The Ewing family of tumors: a subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med 1994, 331: 294–299
24. Panagopoulos I, Mandahl N, Ron D, Höglund M, Nilbert M, Mertens F, Mitelman F, Åman P: Characterization of the CHOP breakpoints and fusion transcripts in myxoid liposarcomas with the 12;16 translocation. Cancer Res 1994, 54:6500–6503
25. Evans HI: Liposarcoma: a study of 55 cases with a reassessment of its classification. Am J Surg Pathol 1979, 3:507–523
26. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989

27. Åman P, Ron D, Mandahl N, Fioretos T, Heim S, Arheden K, Willén H, Rydholm A, Mitelman F: Rearrangement of the transcription factor gene *CHOP* in myxoid liposarcoma with t(12;16)(q13;p11). Genes Chromosomes & Cancer 1992, 5:278–285
28. Knight JC, Renwick PJ, Cin PD, Berghe HVD, Fletcher CDM: Translocation t(12;16) (q13;p11) in myxoid liposarcoma and round cell liposarcoma: molecular and cytogenetic analysis. Cancer Res 1995, 55:24–27
29. Rabbitts TH: Chromosomal translocations in human cancer. Nature 1994, 372:143–149