Jpn. J. Cancer Res. 80, 958–962, October 1989

# Translocation t(12;16)(q13;p11) in Myxoid Liposarcoma of a Child and Implication of the Human *int*-1 Gene in Tumorigenesis

Yuji Fujii,[1] Yuka Matui,[1] Yuichi Nakagawa,[1] Teruaki Hongo,[1] Yoshio Igarashi,[1] Masao Yamada,[2] Yasuo Nakagome[2] and Shigeo Tobayama[3]

[1]*Department of Pediatrics, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu, Shizuoka 431-31,* [2]*National Children's Medical Research Center, 3-35-31, Taishido, Setagaya-ku, Tokyo 154 and* [3]*Division of Pediatric Surgery, Seirei Hamamatsu Hospital, 2-12-12, Sumiyoshi, Hamamatsu, Shizuoka 430*

**Cytogenetic and gene analyses were performed in a child with myxoid liposarcoma (MLS). A reciprocal chromosome translocation t(12;16)(q13:p11) was found in the tumor cells. This result, combined with the previous reports of a similar translocation in adult MLS strongly suggests that this translocation may be a characteristic cytogenetic marker in MLS. The human *int*-1 gene has been reported to be located close to the 12q13 breakpoint associated with MLS. Therefore, we examined the rearrangement of the human *int*-1 gene by Southern blotting analysis. When genomic DNAs from the tumor cells were digested with *Kpn*I, *Eco*RI and *Bam*HI, no difference was seen compared to peripheral blood leukocytes (PBL) DNAs from a normal individual. However, with *Hin*dIII digestion there appeared a 3.1 kb fragment in tumor cell DNA as compared to a 2.8 kb fragment in DNAs prepared from normal PBL and the patient's PBL. These findings suggest that the *int*-1 gene may be implicated in tumorigenesis of MLS with t(12;16)(q13;p11).**

Key words: Myxoid liposarcoma — Chromosomal abnormality — t(12;16)(q13;p11) — Human *int*-1 gene

The recent development of cytogenetic approaches has revealed non-random specific chromosomal changes not only in hematological malignancies but also in solid tumors.[1,2] The relationships between chromosomal breakpoints associated with specific malignant neoplasms and oncogenes have recently been the subjects of intensive investigations.

Liposarcoma is one of the most common malignant mesenchymal neoplasms affecting adults,[3] but is rare in childhood.[4] Recently, a t(12;16)(q13;p11) has been reported in myxoid liposarcoma (MLS), suggesting a specific structural abnormality.[5-8] On the other hand, the human *int*-1 gene has been recently mapped to 12q12-12q13 by using *in situ* hybridization.[9] We report herein a case having MLS with t(12;16) and we present the result of an investigation of possible *int*-1 gene rearrangement in DNA derived from this tumor by Southern blotting analysis.

## MATERIALS AND METHODS

**Case report and histopathologic examination** A 14-year-old Japanese female was admitted to the Seirei Hamamatsu Hospital in July 1984, complaining of abdominal distension which had first been noted six months prior to admission.

Laboratory tests were non-contributory. Angiography and computed tomography showed a huge retroperitoneal tumor. There were no signs of metastasis. A wide surgical resection of the tumor was performed. The tumor exhibited a multilobulated configuration and on section, the soft and sometimes fluctuant tumor frequently had a gelatinous or mucoid appearance. The histopathological examination showed that the tumor consisted of a faintly eosinophilic, homogenous myxoid matrix containing a plexiform network of delicate straight to gently curved capillary-sized vessels, and occasional monovacuolated lipoblasts (signet-ring cells) (Fig. 1). The myxoid matrix of this tumor stained positive with the Alcian blue stain. The diagnosis of myxoid liposarcoma originating in the retroperitoneum was made histopathologically. She was treated subsequently with irradiation to the tumor bed and chemotherapy containing vincristine, actinomycin-D and cyclophosphamide.

After two years, the tumor recurred locally in the abdominal cavity and she underwent complete resection of this tumor in September 1986. Unfortunately she had a second recurrence and again underwent complete resection of the recurrent tumor in October 1987. For the purpose of chromosome and gene analyses, tumor specimens at the second recurrence were immediately transported to our laboratory, Department of Pediatrics, Hamamatsu University School of Medicine. A part of the specimens was stored at $-80^{\circ}C$ until gene analysis was done. The pathological examinations of the first and second recurrent tumors revealed myxoid liposarcoma

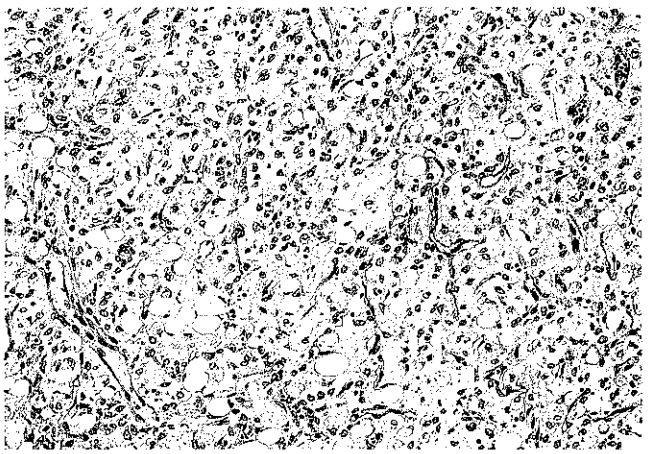

Fig. 1. Light micrograph of the primary tumor showing the typical myxoid liposarcoma appearance with myxoid intracellular matrix, a delicate plexiform capillary network, and monovacuolated lipoblasts (H-E, original magnification × 100).

mimicking the pathological appearance of the primary tumor. As of August 1988 she was alive with a third recurrent tumor.

**Chromosome analysis** The tumor sample was disaggregated mechanically by mincing with scissors and enzymatically digested at 37°C for 6 h with 0.8% collagenase II (Sigma) and 0.002% DNase I (Sigma). The cell suspension was cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum, glutamine and antibiotics. After short-term culture, the cells were treated with colcemid at a final concentration of 0.02 $\mu$g/ml for 6 h. They were then harvested after exposure to 0.075 *M* KCl hypotonic solution for 25 min at 37°C, centrifuged, and fixed in a 3:1 solution of methanol/acetic acid. Slides were stained with the standard trypsin-Giemsa banding technique.

**Gene analysis** High-molecular-weight DNA was prepared from the second recurrent tumor of the patient and peripheral blood lymphocytes (PBL) of the patient and a

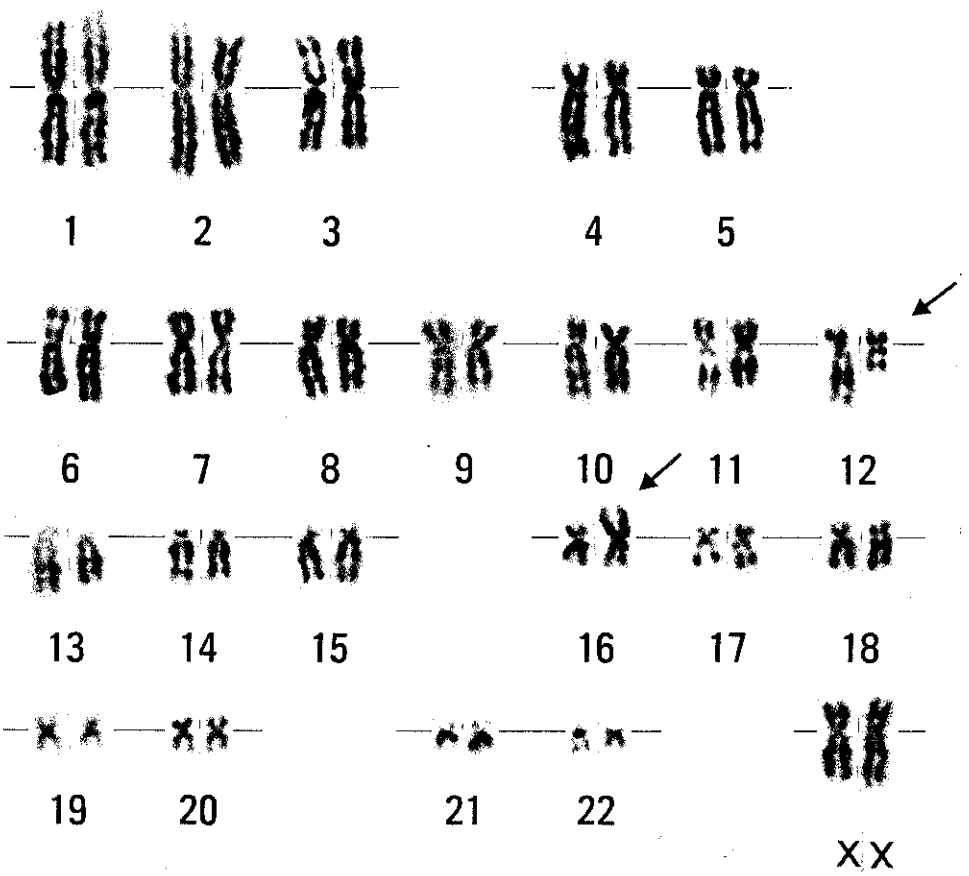


Fig. 2. G-banded karyotype from a tumor cell showing a 46,XX,t(12;16)(q13;p11). (arrows).

normal individual. DNA was evaluated by Southern blotting analysis, using human *int*-1 probe (provided by the Japanese Cancer Research Resources Bank, CO 061). DNA was digested with restriction endonuclease under the conditions recommended by the supplier, and fractionated by electrophoresis using a 0.7% agarose gel. *Kpn*I, *Eco*RI, *Bam*HI and *Hin*dIII were chosen to analyze for the *int*-1 probe. These procedures have been described by Maniatis *et al.*[10]

## RESULTS

**Chromosome analysis** Among 16 metaphases, 14 contained the modal number of 46 chromosomes. The remaining two metaphases showed near-teraploid chromosome counts. All metaphases analyzed were characterized by a reciprocal translocation t(12;16)(q13;p11). No other clonal aberrations, in particular no rings, were found. The karyotype, therefore, was 46,XX,t(12;16)(q13;p11)(Fig. 2 and Fig. 3). Phytohemagglutinin(PHA)-stimulated peripheral blood cells from this patient had a normal karyotype (46,XX).

**Gene analysis** Southern blotting analysis showed hybridization of the human *int*-1 probe to bands of 7.6 kb in *Kpn*I digests, 12.0 kb in *Eco*RI digests, and 10.5 kb in *Bam*HI digests of DNAs from both the tumor specimen and normal PBL. Though the size of the band in *Hin*dIII digests of DNA from normal PBL and the patient's PBL was 2.85 kb, the size from the tumor cells was 3.1 kb, a new single band (Fig. 4).

## DISCUSSION

Although liposarcoma is one of the most common malignant mesenchymal neoplasms affecting adults,[3] it is

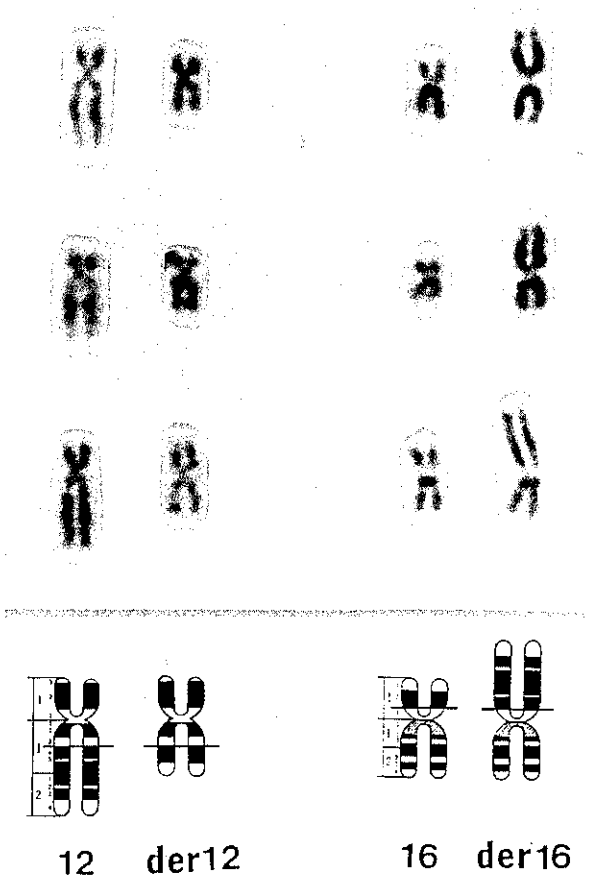


Fig. 3. Partial karyotypes of other tumor cells from this patient (upper three pairs) and schematic presentation (bottom) showing the t(12;16)(q13;p11).

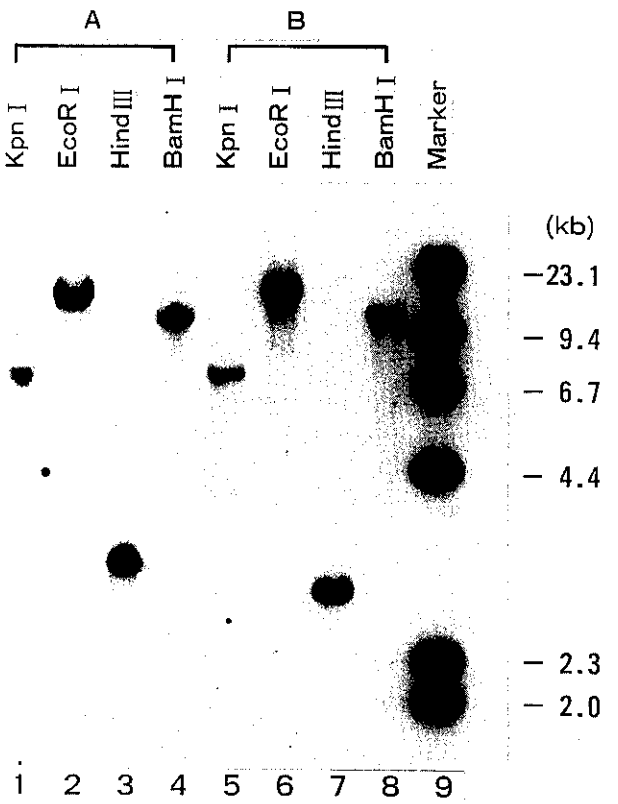


Fig. 4. Representative Southern blotting hybridization of the human *int*-1 gene probe to the tumor cells DNAs (A, lanes 1–4) and normal PBL DNAs (B, 5–8). Hybridization to *Kpn*I-, *Eco*RI-, and *Bam*HI-digested tumor cell DNAs showed no size differences when compared to normal PBL DNAs. Though the size of the band in *Hin*dIII digests of normal PBL DNA (lane 7) was 2.85 kb, there appeared a new band, 3.1 kb in size, in tumor cell DNA (lane 3). The sizes of marker fragments of *Hin*dIII-digested λ DNA are indicated in kilobases (kb).

Table I. Cytogenetic Analysis of Myxoid Liposarcoma (Literature Review)

| Reference | Patient sex[a]/age (yr) | Specimen[b]/site | Chromosome mode | Karyotype |
|---|---|---|---|---|
| Becher *et al.*[11] | M/77 | RT/thigh | near diploid | der(12) |
| Turc-Carel *et al.*[5] | F/47 | PT/thigh | 46 | 46,XX,t(12;16)(q13;p11),t(3;15)(p23;q15) |
| 〃 | M/60 | PT/leg | 46 | 46,XY,t(12;16)(q13;p11),del(6)(q21) |
| 〃 | F/34 | PT/retroperitoneum | 46 | 46,XX,t(12;16)(q13;p11) |
| 〃 | M/65 | RT/thigh | 46 | 46,XY,t(1;12;16)(p11;q13;p11),[loss der(16)] |
| Smith *et al.*[6] | — | Metastasis/— | — | t(12;16)(q13;p11),der(1)t(1;?)(p11 or 13;?),+8, −16 |
| Mertens *et al.*[7] | M/43 | PT/leg | 46 | 46,XY,t(12;16)(q13;p11) |
| Karakousis *et al.*[8] | M/75 | RT/retroperitoneum | 83 | t(12;19)(q13;p or q13),[loss der(12)],del(1)(q11), del(2)(p11),del(3)(p21),6q+,11p+,12p |
| 〃 | M/34 | RT/leg | 46 | 46,XY/46,XY,t(12;16)(q13;p11) |
| Present case | F/17 | RT/retroperitoneum | 46 | 46,XX,t(12;16)(q13;p11) |

*a*) M, male; F, female.
*b*) PT, primary tumor; RT, recurrent tumor.

rare in childhood.[4] Liposarcomas were classified into the following four types on the basis of histopathologic findings by Enzinger and Winslow[3]: liposarcomas of the myxoid cell, round cell, well-differentiated, and pleomorphic types. The myxoid and round cell types were most common in the lower extremity, while the majority of well-differentiated and pleomorphic types originated in the retroperitoneum. There was a marked tendency toward local recurrence in all types. The prognosis was more favorable in patients with myxoid and well-differentiated types than in those with round cell and pleomorphic types.[3]

A consistent chromosomal reciprocal translocation t(12;16)(q13;p11) has recently been described in association with the myxoid type of liposarcoma (MLS).[5-8] To our knowledge, detailed chromosome analyses using banding techniques have been reported in nine MLS cases,[5-8, 11] and so this report is the tenth case (Table I). Our patient is the youngest among the ten reported cases, and also the first case from Japan. Among the reported cases, a common reciprocal translocation, t(12;16)(q13;p11), was found in seven including the present case, a complex t(1;12;16)(p11;q13;p11) in one case,[5] and a t(12;19)(q13;p or q13) in another case.[8] In the case reported by Becher *et al.*,[11] a del(12) was found as one of several abnormalities. A non-random chromosome rearrangement involving chromosome #12, especially at band 12q13, occurs in most of the reported cases. These findings strongly suggested that this rearrangement is of prime importance in the tumorigenesis of MLS.

Quite recently, it appeared that bands 12q13-12q14 were also non-randomly involved in lipoma,[12, 13] the benign counterpart of liposarcoma. The very close proximity of the chromosomal breakpoints 12q13 and 12q14 in MLS and lipoma suggests a common basis for at least one of the possible multiple steps in the genesis of the neoplastic process. It is also suggested that genes crucial for the regulation of cell growth are located in the area 12q13-q14.

The human *int*-1 gene has been assigned, by the use of somatic cell hybrids, to region 12q14 to 12pter on chromosome 12.[14] By *in situ* hybridization, the human *int*-1 gene is now assigned to the segment 12q12-12q13.[9] The function of the human *int*-1 gene product is not yet known. In mammary tumors induced by the mouse mammary tumor virus (MMTV), the mouse *int*-1 gene is frequently activated by an adjacent proviral insertion and is thereby strongly implicated in tumorigenesis.[15-17] Brown *et al.*[18] recently reported that a retroviral vector expressing the *int*-1 caused partial transformation of a mammary epithelial cell line and therefore, the *int*-1 gene was a novel proto-oncogene with the potential to contribute to neoplasia. The mouse and human *int*-1 gene have a high level of homology.[19] Therefore, it is of interest to determine if the human *int*-1 gene is rearranged in DNA of MLS carrying the t(12;16)(q13;p11). Hybridization to *Kpn*I-, *Eco*RI- and *Bam*HI-digested tumor cell DNAs showed no size differences when compared to normal PBL DNAs. But hybridization to *Hin*dIII-digested tumor cell DNA showed a new band of molecular weight 3.1 kb as compared to DNA from normal PBL of 2.85 kb. These findings suggest that the *int*-1 gene may be implicated in tumorigenesis of MLS with t(12;16)(q13;p11). Though these data are in contrast to the report by Turc-Carel *et al.*[9] that the *int*-1 gene was not rearranged in two tumors, we do not know the reason for this

discrepancy. As regards the possible involvement of the *int*-1 gene in MLS with t(12;16)(q13;p11), we need to investigate further cases, not only at the DNA level but also at the RNA level.

ACKNOWLEDGMENTS

We thank Dr. Toshiya Inaba and Mr. Toshiro Nishida for helpful comments on the cytogenetics.

*(Received May 17, 1989/Accepted July 31, 1989)*

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