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ORIGINAL ARTICLE

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Establishment and characterization of a cell line from a malignant fibrous histiocytoma of bone developing in a patient with multiple fibrous dysplasia

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Abstract *Purpose and methods*: In order to provide material for genetic analysis of fibrous dysplasia (FD), a cell line designated GBS-1 was established from a secondary bone malignant fibrous histiocytoma (MFH) developing in a rib of a 44-year-old male polyostotic FD patient. *Results*: The GBS-1 cells are characterized by a pleomorphic spindle cell morphology with abundant mucus production. On transplantation to nude mouse subcutis the cell line forms myxoid-spindle cell sarcomas with giant cells, the myxoid product being positive for periodic acid-Schiff (PAS) and alcian blue (Al-B) stains and completely digested by hyaluronidase, mimicking the original tumor. Chromosome and genetic analyses revealed multiple structural and numerical abnormalities of chromosomes with a large number of unidentifiable chromosomes and p53 mutation in exon 7 with LOH in the counterpart. *Conclusions*: Since cell lines for FD have hitherto not been available, the GBS-1 cells should prove useful for genetic analyses of FD and also MFH of bone origin.

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Keywords Bone · Malignant fibrous histiocytoma (MFH) · Fibrous dysplasia (FD) · Cell line · Chromosomal analysis

Introduction

Fibrous dysplasia (FD) of bone is a benign lesion characterized by proliferation of fibrous connective tissue associated with metaplastic formation of immature non-lamellar bone. It has long been considered a developmental disorder. However, recent reports showed clonal growth of cells with chromosomal aberrations in FD, suggesting a possible neoplastic character (Bridge et al. 1989; Tarkkanen et al. 1993; Mertens et al. 1994; Dal Cin et al. 2000). The real nature of FD, especially regarding genetic and/or epigenetic changes remains to be elucidated.

In vitro culture systems have distinct advantages for analytical studies. However, attempts in various laboratories, including our own, to establish culture cell lines from FD have not been successful. We therefore have been trying, as a second-best strategy, to obtain culture lines from secondary sarcomas developing with a background of FD. Secondary sarcomas, including osteosarcomas, fibrosarcomas chondrosarcomas, and malignant fibrous histiocytomas (MFHs) are a rare finding in FD (Ruggieri et al. 1994). The present paper describes establishment of an MFH cell line derived from a rib FD lesion in a polyostotic, polymeric FD patient. To the best of our knowledge, this is the first such cell line. Its unique MFH characteristics, including hyaluronic acid (HA) production and histological patterns when grown in nude mice, indicate that it might prove to be a good material for analysis of FD and also MFH of bone.

Materials and methods

Original tumor

A 44 -year-old man developed a rapidly growing tumor in a preexisting FD of the left 11th rib. The patient had a history of surgery

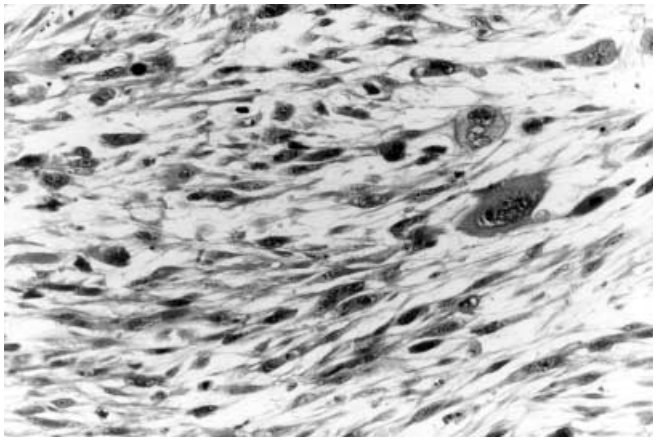

Fig. 1 Histology of the original tumor (HE staining). Pleomorphic and spindle cells are proliferating with high cellularity. Note the mitoses and giant cells

for correction of skull and nasal bone deformities due to FD at the ages of 15 and 25, respectively. Details of this surgery was reported previously (Nakamura et al. 1981). He had no history of radiation therapy. Clinical investigations revealed multiple other bone lesions involving the skull, right pelvis, right femur, right tibia, and bilateral ribs, all FD on image diagnosis. A biopsy of the growing rib tumor revealed an MFH comprising pleomorphic spindle cells with marked myxoid change and many giant cells (Fig. 1). After preoperative chemotherapy and radiotherapy, the rib tumor, sized 16×12×9 cm was widely excised, followed by postoperative chemotherapy. The patient died 13 months after the operation with multiple lung metastases.

Immunohistochemical study for FD and MFH

p53, MDM 2, and Ki-67 immunohistochemistry was conducted as described previously (Goto et al. 1998) for biopsy material of original tumor (MFH) and FD sample removed at the age of 25 (Nakamura et al. 1981).

Establishment of the cell line

The MFH tissue material removed at biopsy, prior to chemotherapy, was finely minced and seeded onto 60-mm culture dishes (Greiner, Germany) supplied with RPMI1640 medium supplemented with 10% fetal calf serum (GIBCO, Great Island, N.Y. USA). After 1 week of culture, the first passage was made and chromosomal analysis was performed. Passages were accomplished using trypsin (GIBCO) and culture medium was changed to DMEM after 6 months. The cells were subcultured once a week thereafter and at 80 passages the cell line was designated GBS-1 and underwent characterization.

Chromosome analysis

Cells were treated with 0.1 μg per millilitre colcemid (GIBCO) for 2 h, then lysed with 0.05 M KCl for 30 min and fixed with methanol-acetic acid (3:1). Chromosome preparations were stained with quinacrine and numbers counted for ten metaphase nuclei.

Growth curve

Cells were seeded onto 60-mm dishes, 1×10^6 cells each, containing 2 ml DMEM medium supplemented by 10% fetal calf serum and harvested with 0.25% trypsin every 24 h. Viable cells, determined by the trypan blue exclusion test, were counted using a hemocytometer, and the doubling time was estimated in the logarithmic growth phase.

Tumorigenicity

Three 6-week-old athymic nude mice (Nihon Clea, Tokyo, Japan) were inoculated subcutaneously with 1×10^7 cells and maintained in isolated sterile cages.

HE, periodic acid-Schiff (PAS), alcian-blue (Al–B), and Hyaluronidase-digested Al–B staining

Formalin-fixed paraffin embedded tissue samples were deparaffinized and 4-μm sections were stained with HE, PAS, and Al-B, routinely.

Hyarulonidase-digestion and Al-B staining were performed with 50 mg/l hyaluronidase (Sigma, St. Louis).

Immunohistological study for transplant tumor

HMG-1, Muc2, and CA15–5 immunohistochemistry was conducted as described earlier (Yamato et al. 1999). After deparaffinization and re-hydration, 4-μm formalin-fixed, paraffin-embedded sections of transplant tumors were exposed to anti-human gastric mucin (NCL-HGM-45M1, Novo Castra, Newcastle, UK), Muc-2 glycoprotein (NCL-MUC-2, Novo Castra) or CA15–5 (DF-3, Centocor, USA) primary antibodies at dilutions of 1:50, 1:100, and 1:1, respectively. Subsequent procedures with the streptavidin-biotin-peroxidase (SAB) system (Histofine SAB-PO kit, Nichirei Corp) were as previously detailed (Goto et al. 1998).

PCR SSCP for p53 gene mutation

Genomic DNA was extracted from the cell line and transplanted tumor by the SDS-proteinase K method (Kanda et al. 1993). Highly conserved regions of the p53 gene, in exons 5, 6, 7, and 8, were examined by SSCP using the primers of Miyaki et al. (Miyaki et al. 1993). PCR products were electrophoresed and autoradiographed as described previously (Wada et al. 1999). Shifted bands were cut out and DNA was extracted and subcloned into the pGEM-T easy vector (Promega, Madison, Wis., USA) following the manufacturer's protocol, then sequenced using a cycle-sequence kit (Amersham-Pharmacia).

LOH study for p53 gene

Control DNA was extracted from the formalin fixed, paraffin embedded muscle tissue removed at wide resection of original tumor by the method of Goelz et al. (Goelz et al. 1985). Microsatellite primers of HSGPOCA3 and TP53 were used for analysis of LOH at 17p13 (p53) locus (Jones et al. 1994). Details were described previously (Goto et al. 1998).

Results

Immunohistochemical study for FD and MFH

Over 90% and about 10% of MFH tumor cells showed strongly positive for p53 and Ki-67, respectively. There were no p53- nor Ki-67-positive cells in FD. MDM2 was negative in both MFH and FD.

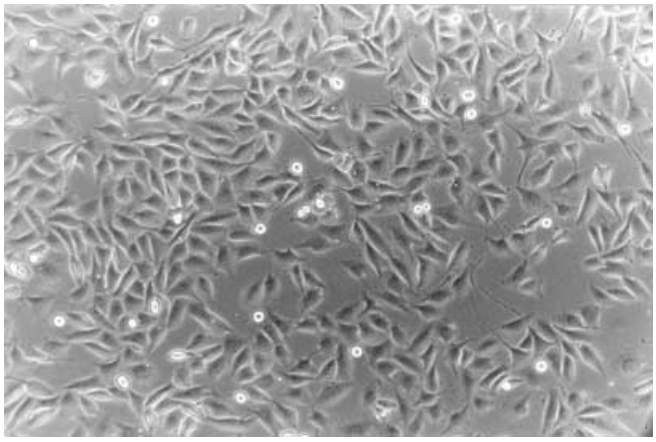

Fig. 2 Phase contrast appearance of GBS1. Spindle-shaped or polymorphic cultured cells are illustrated

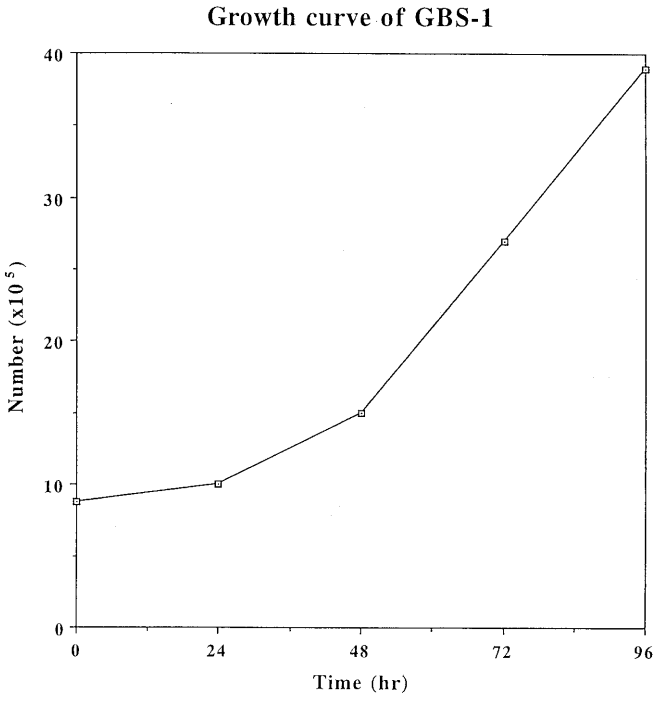

Fig. 3 Growth curve. The cells entered the logarithmic growth phase at around 48 h, the population doubling time estimated during this phase being about 30 h

Cultured cells

GBS-1 cells were polymorphic or spindle-shaped. Mitoses were frequent. Representative features under phase-contrast microscopy are illustrated in Fig. 2. The cells produced viscous material. A typical growth curve is shown in Fig. 3. The cells entered logarithmic growth phase at 48 h, with a population doubling time estimated during this of about 30 h.

Chromosomal analysis

A representative karyotype is shown in Fig. 4. The tumor has near-diploid and near-tetraploid clones with multiple numerical and structural aberrations. Although clonal structural abnormalities such as del(1)(p13) and five marked chromosomes of unknown origin (mar1–mar5: bottom in Fig. 4) were identified, about half of chromosomes were nonclonal markers. Cytogenetically, this karyotype was characterized by complex changes and considerable heterogeneity. Composite and incom-

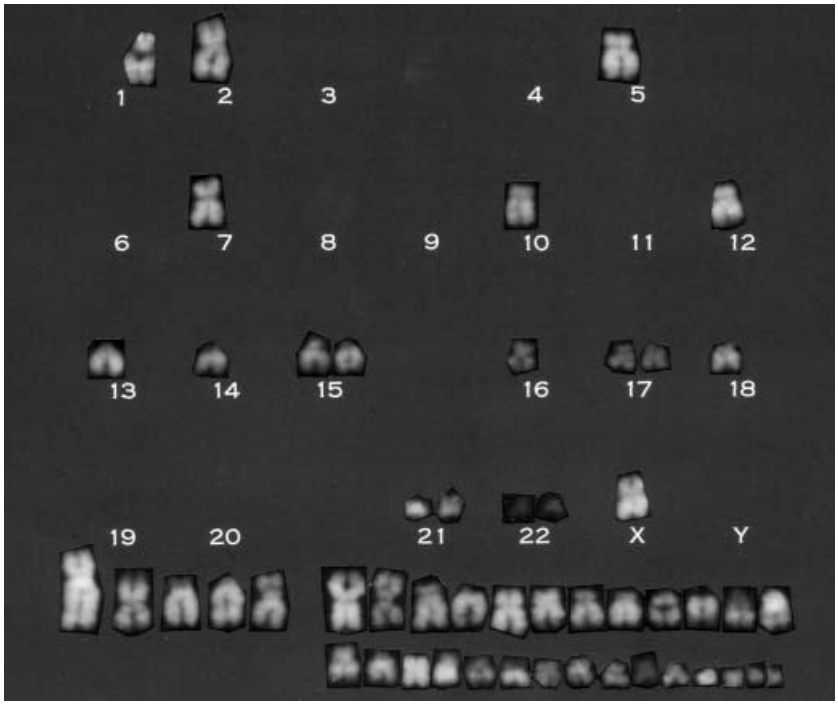

Fig. 4 Chromosomal analysis. Q-banded karyotype of the near-diploid cell containing a large number of chromosomal aberrations. Most of chromosomes are structurally rearranged, with marker chromosomes seen at the bottom. The tumor has the karyotype 43–52, X, –Y, del(1)(p13), +mar1, +mar2, +mar3, +mar4, +mar5, +19–27mar, inc[cp7]/ 91–101, idem x2[cp2]

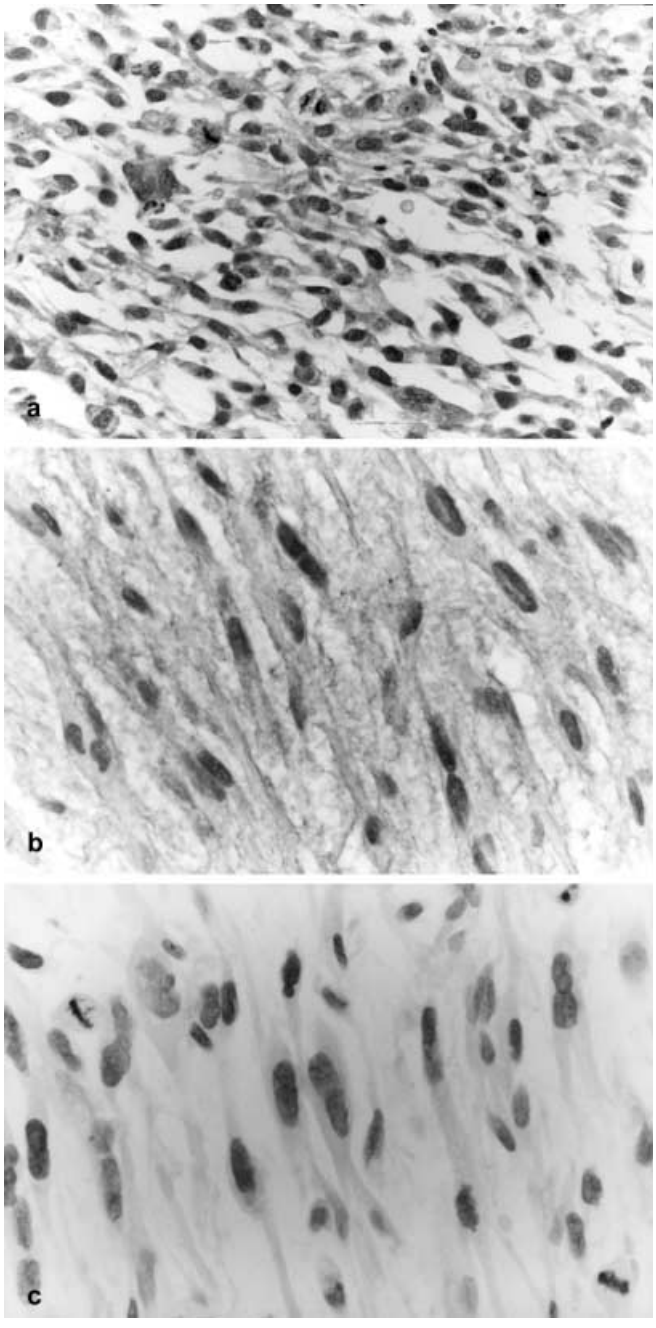

Fig. 5a–c Histology of a nude mouse transplant tumor. **a** HE staining (original ×50). Spindle cells with pleomorphism and abundant myxoid substance are evident in a nude mouse transplanted tumor. Giant cell formation is apparent and mitoses are frequent. **b** Alcian-blue staining (original 200×). Abundant Al-B positive myxoid substance is apparent around and in the tumor cells. **c** Hyaluronidase-digested alcian-blue (original 200×). The myxoid substance shown in Fig. 5b is completely absent after hyaluronidase treatment

plete karyotype was described as follows: 43–52, X, –Y, del(1) (p13), +mar1, +mar2, +mar3, +mar4, +mar5, +19–27mar, inc[cp7]/ 91–101, idem ×2[cp2]. No homogeneously-staining region (hsr) could be identified.

Detection of p53 gene mutations and LOH

An abnormally shifted band without the normal counterpart could be identified by PCR-SSCP. Sequencing analysis showed a point mutation in exon 7 [Codon 312, TTC(Phe) to TTA (Leu)]. Since normal control tissue (muscle) showed only TTC, it was thought of as somatic mutation.

This patient was heterozygous for both HSGPOCA and TP53 (p53) loci. GBS-1 cell line show LOH in both loci.

Transplantation into nude mice

After inoculation of cells into the subcutis of nude mice, approximately bean-sized tumors became palpable after 2 weeks in all cases, these growing to 3×3 cm in size after 3 months. The typical histopathological appearance is shown in Fig. 5a. Principally they comprised pleomorphic spindle cells with eosinophilic cytoplasm intermingled with multinuclear giant cells. Characteristically they produced abundant myxoid substance, positive for PAS, Al-B (Fig. 5b), but negative for organ specific HGM, Muc2, or CA15–5 mucins. The Al-B positive substance was completely digested by hyaluronidase (Fig. 5c).

Genetic change of the nude mouse tumor

Chromosomal analysis of nude mice tumor exhibited a more complex karyotype with a chromosome number in the hypertriploid to hypertetraploid range; no near-diploid clone was observed: 74[1]/77[2]/79[1]/81[1]/82[1] 84[1]/85[2]/86[2]/88[1]/89[1]90[2]/91[1]92[2]/94[1]. 77–94, X, –Y, del(1)(p13), +51–59mar, inc[cp5], in which a characteristic change in original tumor of del(1)(p13) was maintained, that confirmed the nude mice tumors to be derived from GBS-1. In addition, sequencing and LOH analyses showed p53 gene mutation in exon 7 with LOH was maintained in nude mice tumor.

Discussion

For a long period after Lichtenstein's first description of FD in 1938, the ailment was basically regarded as a form of dysplasia and not a neoplasia. However, since 1989, several cytogenetical investigations have revealed features of clonal proliferation with chromosomal aberrations (Bridge et al. 1989; Tarkkanen et al. 1993; Mertens et al. 1994; Bridge et al. 1999; Dal Cin et al. 1999) and recently, the CHAMP study group summarized the reported cases with their original analyses and concluded that FD is a neoplastic disease associated with genetic lesions in chromosomes 2 and 12p13 (Dal Cin et al. 2000). Thus, identification of the responsible genes has become a current focus of interest, although the true nature of FD remains equivocal.

The GBS-1 established in the present work is characterized by marked karyotypical abnormalities with many marker chromosomes. However, aberrations of chromosomes 2 or 12p13 were not identified. In addition, the homogeneously-staining region (hsr), reported to be frequent in bone MFH (Sandberg and Bridge 1994) was also not detected. On the other hand, a p53 gene mutation with loss of the normal allele was found. Since no p53 inactivation has previously been detected in FD, it may have occurred during progression from FD to MFH or after. Although p53 is frequently mutated in bone sarcomas (up to 25%), it was apparent in only 10.5% of one series of bone MFHs (Taubert et al. 1998).

While MFH is the most common soft-part sarcoma of adults it is rare in bone, where it accounts for less than 2% of all malignancies, most frequently located in the long bones of the extremities. Since the recognition of bone MFH as a distinct bone tumor in 1972 (Feldman and Norman 1972), its characteristics have been well analyzed in comparison with counterpart lesions of soft tissue: 1) bone MFH occurs at a younger age; 2) predominant subtypes differ between them, especially, the myxoid subtype being rare in bone MFH (Yokoyama et al. 1993); and 3) secondary MFH, especially post bone-infarction or post-irradiation, is frequent (30%) in bone MFH, such episodic occurrence being rare with soft-tissue MFH (Yokoyama et al. 1993). Although many soft-tissue MFH cell lines have been established, there is only one cell line of a bone MFH reported so far [NMSG 10, (Yokota et al. 1995)]. Like the present GBS-1, the reported cell line produced HA markedly. Alcian-blue -positive substance was characteristic and diagnostic for MFH. The main component of this alcian-blue positive substance is thought of as HA. As discussed by Nishikawa et al., HA production is characteristic in MFH cell line, too, regardless of bone or soft-part origin. Genetic changes have so far been not reported concerning NMSG10.

Culture systems have many advantages for examination of genetic and biological characteristics. Our GBS-1 cell line might thus prove useful for assessment of genetic changes of FD and also MFH of bone.

This GBS-1 is available from the corresponding author. E-mail to: kandah@jfcr.or.jp.

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