Establishment and Characterization of a New Cell Line, FPS-1, Derived from Human Undifferentiated Pleomorphic Sarcoma, Overexpressing Epidermal Growth Factor Receptor and Cyclooxygenase-2

MICHIYUKI HAKOZAKI[1,2], HIROSHI HOJO[1], MICHIKO SATO[1], TAKAHIRO TAJINO[2], HITOSHI YAMADA[2], SHINICHI KIKUCHI[2] and MASAFUMI ABE[1]

[1]First Department of Pathology and [2]Department of Orthopaedic Surgery, Fukushima Medical University School of Medicine, Fukushima, Japan

Abstract. *Background: Undifferentiated pleomorphic sarcoma (UPS) is among the most common soft tissue sarcomas in adults. In order to improve its aggressive course or prognosis and establish new therapeutic methods, molecular genetic and biological characterizations of UPS are required. Materials and Methods: A new human UPS cell line (FPS-1) was established from UPS of the upper arm of a 79-year-old man. The cell line has been maintained for over 14 months with more than 60 passages. FPS-1 cells were characterized using molecular biological methods. Results: FPS-1 cells showed the same morphological and immunophenotypical characteristics as the primary tumor. Cytogenetic and molecular analyses revealed a nonsense mutation in exon 6 of the p53 gene. Epidermal growth factor receptor (EGFR) and cyclooxygenase-2 (COX-2) were expressed in FPS-1 cells. Conclusion: FPS-1 cells might be useful for investigating biological behavior and developing new molecular targeting antitumor drugs for UPS with EGFR or COX-2 expression.*

The term malignant fibrous histiocytoma (MFH) is widely used to describe pleomorphic soft tissue sarcomas which do not exhibit a specific line of differentiation. MFH is included in the category of fibrohistiocytic soft tissue tumors. Recently, MFH has been defined as one of the sarcomas included in "undifferentiated (high-grade) pleomorphic sarcoma (UPS)" in the newest World Health Organization (WHO) classification of soft tissue and bone tumors (1). UPS is one of the most common soft tissue sarcomas in adults, but the origin of these tumor cells remains obscure. UPS shows a broad range of histologic features and is divided into several subtypes: storiform-pleomorphic (ordinary), myxoid, giant cell and inflammatory (1, 2).

UPS frequently shows highly aggressive behavior, resistance to multi-agent chemotherapy and fatal metastasis. Therefore, to improve the aggressive course or prognosis and to develop new therapeutic methods, especially antitumor drugs, molecular genetic and biological characterization of UPS are required.

A number of MFH cell lines have been reported (3-21). However, these cell lines have not been adequately investigated from the standpoint of their molecular genetic and biological behavior. In the present study, we aimed to establish and characterize a new cell line, FPS-1, derived from UPS (storiform-pleomorphic MFH). The overexpression of epidermal growth factor receptor (EGFR) and cyclooxygenase-2 (COX-2) on FPS-1 was investigated to clarify the biological behavior of UPS.

Materials and Methods

Case report. The patient was a 79-year-old Japanese man. He underwent total gastrectomy for gastric carcinoma at the age of 66 and a right colectomy for ascending colonic mucinous adenocarcinoma at the age of 78. The patient was admitted to our hospital with a 10-month history of slowly increasing mass and slight motional pain in his right upper arm. The patient had received magnetic resonance imaging (MRI) of his right upper arm, revealing a 77 x 68 x 45 mm intramuscular tumor in the biceps brachii. MRI showed low intensity on the T1-weighted image, high intensity on the T2-weighted image and diffuse enhancement on a Gd-enhanced T1-weighted image. No distant metastasis was found on radiological analysis. A primary malignant soft tissue tumor was suspected and an open biopsy was performed. Light microscopy of the biopsy specimens from the right upper arm tumor showed diffuse proliferation of spindle-shaped or pleomorphic cells arranged in a storiform pattern, and anaplastic uninucleated giant cells were

Correspondence to: Michiyuki Hakozaki, MD, First Department of Pathology, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima-shi, Fukushima 960-1295, Japan. Tel: +81 24 547 1165, Fax: +81 24 548 4488, e-mail: paco@fmu.ac.jp

Key Words: Undifferentiated pleomorphic sarcoma, malignant fibrous histiocytoma (MFH), FPS-1 cell line, p53 gene, epidermal growth factor receptor (EGFR), cyclooxygenase-2 (COX-2), sarcoma.

scattered (Figure 1). The spindle-shaped and pleomorphic cells had atypical nuclei, and mitotic figures were frequently seen. Immunohistochemically, the tumor cells reacted positively to vimentin and lysozyme (Table I), and the tumor was consistent with UPS. After radiation therapy (X-ray, total 30.8 Gy), a marginal resection was performed and small numbers of viable cells were found in the resected tissue. At the time of writing, the patient is alive, with no sign of local recurrence or metastasis in 12 months of postoperative follow-up time.

Establishment of cell line. Tumor cells were obtained from the biopsy specimen. The cells were cultured at an initial concentration of $1.0x10^6$ viable cells/ml in RPMI-1640 medium (Sigma R8758, St. Louis, MO, USA) supplemented with 15% heat-inactivated fetal calf serum (FCS) (JRH Biosciences, Lenexa, KS, USA), 50 units/ml penicillin G and 50 μg/ml streptomycin. They were inoculated into 25-cm^2 tissue culture flasks (Iwaki Glass, Tokyo, Japan) and incubated at 37˚C in a humidified atmosphere with 5% CO_2. The culture medium was changed twice per week. To harvest and transfer the cell line, the cells were treated with 1,000 unit/ml Dispase (Sanko-Junyaku, Tokyo, Japan) when in a sub-confluent state. The cell line has been maintained for over 14 months under these culture conditions.

Morphological study. The growth and morphology of cultured cells *in vitro* were observed with an inverted microscope. Morphological characteristics were further determined by May-Giemsa staining on cytospin preparations and by hematoxylin-eosin staining on paraffin-embedded sections.

Immunohistochemistry. Immunohistochemical analysis was performed on the primary tumor, cultured cells and heterotransplanted tumors using the streptavidin-biotin complex (SABC) method. The antibodies were as follows: vimentin (clone V9, 1:1000 dilution; Dako, Glostrup, Denmark), desmin (clone D33, 1:20 dilution; Dako), α-smooth muscle actin (α-SMA; clone 1A4, 1:100 dilution; Dako), caldesmon (clone h-CD, 1:50 dilution; Dako), S-100 protein (polyclonal, 1:2,000 dilution; Dako), cytokeratins including AE1/AE3 (clone AE1 and AE3, 1:200 dilution; Dako), CD34 (clone NU-4A1, 1:50 dilution; Nichirei, Tokyo, Japan), CD68 (clone KP1, 1:100 dilution; Dako), CD99 (MIC-2; clone 12E7, 1:200 dilution; Dako), lysozyme (polyclonal, 1:2,000 dilution; Dako), α-1-antitrypsin (AT; polyclonal, 1:10,000 dilution; Dako), Ki-67 (MIB-1; clone MIB-1, 1:300 dilution; Dako), p53 gene product (clone DO-7, 1:150 dilution; Dako), EGFR (clone EGFR.25, 1:100 dilution; Novocastra, Newcastle-upon-Tyne, UK) and COX-2 (clone 4H12, 1:100 dilution; Novocastra).

Cell population doubling time. In order to determine the doubling time, $3.5x10^5$ cells at the 45th passage were seeded on 3.5 cm plastic dishes (BD Falcon 353046, BD Biosciences, Franklin Lakes, NJ, USA) with fresh culture medium containing 15% FCS. The dishes were harvested and the number of viable cells in each dish was counted in accordance with the dye exclusion test (0.1% trypan blue in phosphate-buffered saline; PBS) every 24 h for 7 days.

Heterotransplantation. Severe combined immunodeficiency (SCID) mice (lcr/scid female) and athymic nude mice (BALB/cA-nu/nu female) were purchased at 6 weeks of age from CLEA Japan (Tokyo, Japan) and kept under sterile conditions. The animal experiments were carried out under the control of our committee in accordance with the guidelines on Animal Experiments at Fukushima Medical University, the Japanese Government Animal Protection and Management Law (No. 105) and the Japanese Government Notification of Feeding and Safekeeping of Animals (No. 6). The mice were given a single subcutaneous injection of $5x10^6$ cells at the 15th passage from the cultured cells in the log phase of growth.

Table I. *Immunohistochemical reactivity of primary tumor, FPS-1 cell line (in vitro), and heterotransplanted tumor (nude mouse; in vivo) with various antibodies.*

Antibodies	Primary tumor	FPS-1 (*in vitro*)	FPS-1 (*in vivo*)
Vimentin	4+	4+	4+
Desmin	–	–	–
α-SMA	–	–	1+
Caldesmon	–	–	–
S-100 protein	–	–	–
AE1/AE3	–	–	–
CD34	–	–	–
CD68	–	3+	±
CD99 (MIC-2)	–	1+	–
Lysozyme	4+	4+	4+
AT	–	–	2+
p53	2+ (26.0%)	1+ (7.4%)	–
Ki-67 (MIB-1)	3+	3+	4+
EGFR	4+	4+	4+
COX-2	4+	4+	4+

–, negative; ±, rare cell positive (<1% positive cells); 1+, <10% positive cells; 2+, 10-50% positive cells; 3+, 50-90% positive cells; 4+, >90% positive cells.

Cytogenetics. Karyotype analysis of FPS-1 cells at the 39th passage was performed commercially by SRL (Tokyo, Japan) using the trypsin G-banding technique.

DNA sampling. DNA samples were extracted from FPS-1 cells at the 40th passage using Sepagene (Sanko-Junyaku) according to the manufacturer's protocol.

Polymerase chain reaction – single strand conformational polymorphism (PCR-SSCP) and direct sequencing analysis for p53 gene. Genomic PCR-SSCP in p53 gene exons 5 to 8, which are considered to be this gene's mutational hot spot, was performed on FPS-1 cells at the 32nd passage (SRL). Moreover, oligonucleotide sequences in exons 5 to 9 of the p53 gene were analyzed by direct sequencing. Using Takara Ex Taq HS polymerase (Takara Bio, Shiga, Japan), 0.5 μg of DNA was amplified in a total volume of 50 μl. The sequencing primers were as follows (22, 23): exons 5-6 (sense): 5'TTCCTCTTCCTGCAGTACTC-3'; exons 5-6 (antisense): 5'-AGTTGCAAACCAGACCTCAG-3'; exon 7 (sense): 5'-GTGTTGCCTCCTAGGTTGGC-3'; exon 7 (antisense): 5'-CAAGTGGCTCCTGACCTGGA-3'; exons 8-9 (sense): 5'-CCTA TCCTGAGTAGTGGTAA-3'; exons 8-9 (antisense): 5'-CCAAGA CTTAGTACCTGAAG-3'. These primers were obtained from Sigma-Aldrich Japan (Hokkaido, Japan). Polymerase chain reaction (PCR) was performed as follows: denaturing at 94˚C for 10 min followed by 30 cycles of amplification (94˚C for 1 min, 60˚C for 1 min, 72˚C for 1 min) and a 10-min extension at 72˚C in a thermal cycler (i-cycler, Bio-Rad Laboratories, Hercules, CA, USA). One microliter of the PCR product was applied for PCR under conditions

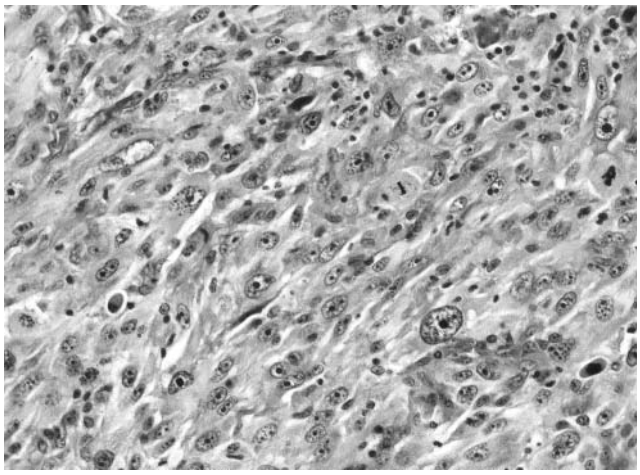

Figure 1. *Histology of the primary right upper arm tumor. The tumor cells are diffusely proliferate and are spindle-shaped or pleomorphic cells arranged in a storiform pattern, accompanied by scattered anaplastic uninucleated giant cells. Mitotic figures are frequent.*

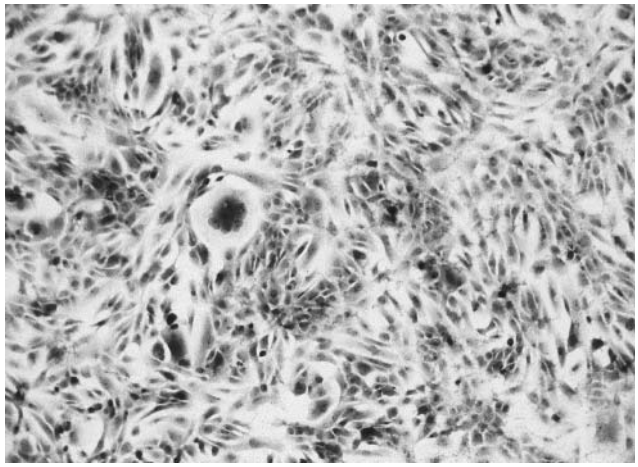

Figure 2. *Inverted microscopy of FPS-1 cells in vitro. The adherent cultured cells are atypical fibroblast-like spindle-shaped or histiocyte-like polygonal-shaped without contact inhibition, and uninucleated giant cells are also present.*

consisting of 1 cycle of 95˚C for 5 min and 25 cycles of 95˚C for 30 sec and 60˚C for 30 sec by the direct sequencing method (Thermo Sequenase core sequencing kit with 7-deaza-dGTP; Amersham, Cleveland, OH, USA). Subsequently, oligonucleotide sequences of exons 5 to 9 of the p53 gene were analyzed using a sequencer (SQ-5500, Hitachi, Tokyo, Japan) and compared with the germline sequences recorded in the GenBank database.

RNA preparation and reverse transcription-PCR (RT-PCR) analysis for EGFR and COX-2 mRNA expression. Total RNA from FPS-1 cells at the 47th passage was isolated using ISOGEN reagent (Wako Pure Chemical Industries, Osaka, Japan). After priming of 5 μg of total RNA with 1 μl of 1,000 pmol random hexadeoxynucleotide primers (Takara Bio) and 1 μl of 10 mM dNTP mixture (Takara Bio), reverse transcription was performed using SuperScript III RNase H- Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Aliquots of cDNA (1 μl) were amplified using Takara Ex Taq HS polymerase in a total volume of 50 μl. For detection of EGFR and COX-2 mRNA, PCR was performed in a thermal cycler. A housekeeping gene, β-actin (24), was used as an internal control. PCR for EGFR and β-actin was performed as follows: denaturing at 94˚C for 10 min followed by 35 cycles of amplification (94˚C for 1 min, 56˚C for 1 min, 72˚C for 1 min) and 10-min extension at 72˚C (25); and for COX-2, denaturing at 95˚C for 5 min followed by 40 cycles of amplification (94˚C for 20 sec, 59˚C for 1 min) and 10-min extension at 59˚C (26). The sequencing primers were as follows: 5'-CTATGAGATGGAGGAAGACG-3' (sense) and 5'-CAGAGGAGGAGTATGTGTGA-3' (antisense) for EGFR (25), 5'-ATGGAATTACCCAGTTTGTTGAATC-3' (sense) and 5'-TGGA AGCCTGTGATACTTTCTGTACT-3' (antisense) for COX-2 (26), and 5'-GACTATGACTTAGTTGCGTTA-3' (sense) and 5'-GCCT TCATACATCTCAAGTTG-3' for β-actin (24). These primers were obtained from Sigma-Aldrich Japan. PCR reaction products were electrophoresed through 2% agarose gels containing 0.2 mg/ml ethidium bromide. The FRTK-1 cell line, derived from human malignant rhabdoid tumor of the kidney and established in our laboratory (27), was used as a positive control for EGFR and COX-2.

Western blotting analysis for EGFR and COX-2. FPS-1 cells at the 46th passage, grown in a 25-cm^2 tissue culture flask, were washed twice with ice-cold 0.01 M PBS and scraped in ice-cold 0.01 M PBS. The cell pellets were lysed in 1 ml of lysis buffer (20 mM Tris-HCl pH 7.4, 1% NP-40, 1 mM EDTA, 50 mM NaF, 50 mM beta-glycerophosphate, 0.05 mM Na_3VO_4, 0.1 mM PMSF, 0.01 mg/ml aprotinin, 0.01 mg/ml leupeptin, 0.01 mg/ml pepstatin) at 4˚C for 1 h and were clarified by centrifugation (1,200 rpm for 15 min). Subsequently, 12 μl of the supernatant was suspended in 3 μl of sample buffer containing 62.5 mM Tris-HCl pH 6.8, 10% glycerol, 5% 2-mercaptoethanol and 2.3% SDS. The samples were subjected to SDS-PAGE (7.5% of polyacrylamide gel for EGFR and 13% of polyacrylamide gel for COX-2) under reducing conditions at a constant current of 20 mA. The separated proteins were transferred to PVDF membrane (Millipore, Tokyo, Japan) at a constant current of 300 mA for 60 min. To block non-specific binding, the membrane was incubated with blocking buffer (Pierce, Rockford, IL, USA) according to the manufacturer's protocol. The membrane was washed with washing buffer and incubated with the antibodies of EGFR (1005; sc-03, 1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and COX-2 (H-62; sc-7951, 1:200 dilution; Santa Cruz Biotechnology). After washing, the membrane was incubated with 1 μg/ml anti-mouse or anti-rabbit immunoglobulin (Bio-Rad Laboratories). The proteins were visualized using an ECL Western blotting detection reagent and analysis system (Amersham). The FRTK-1 cell line was used as a positive control for EGFR and COX-2.

Results

Immunohistochemical analysis for p53, EGFR and COX-2 on primary tumor cells. The tumor cells showed strong immunopositivity for EGFR and COX-2 (Table I), and focal immunopositivity for p53 (approximately 26.0% positive cells).

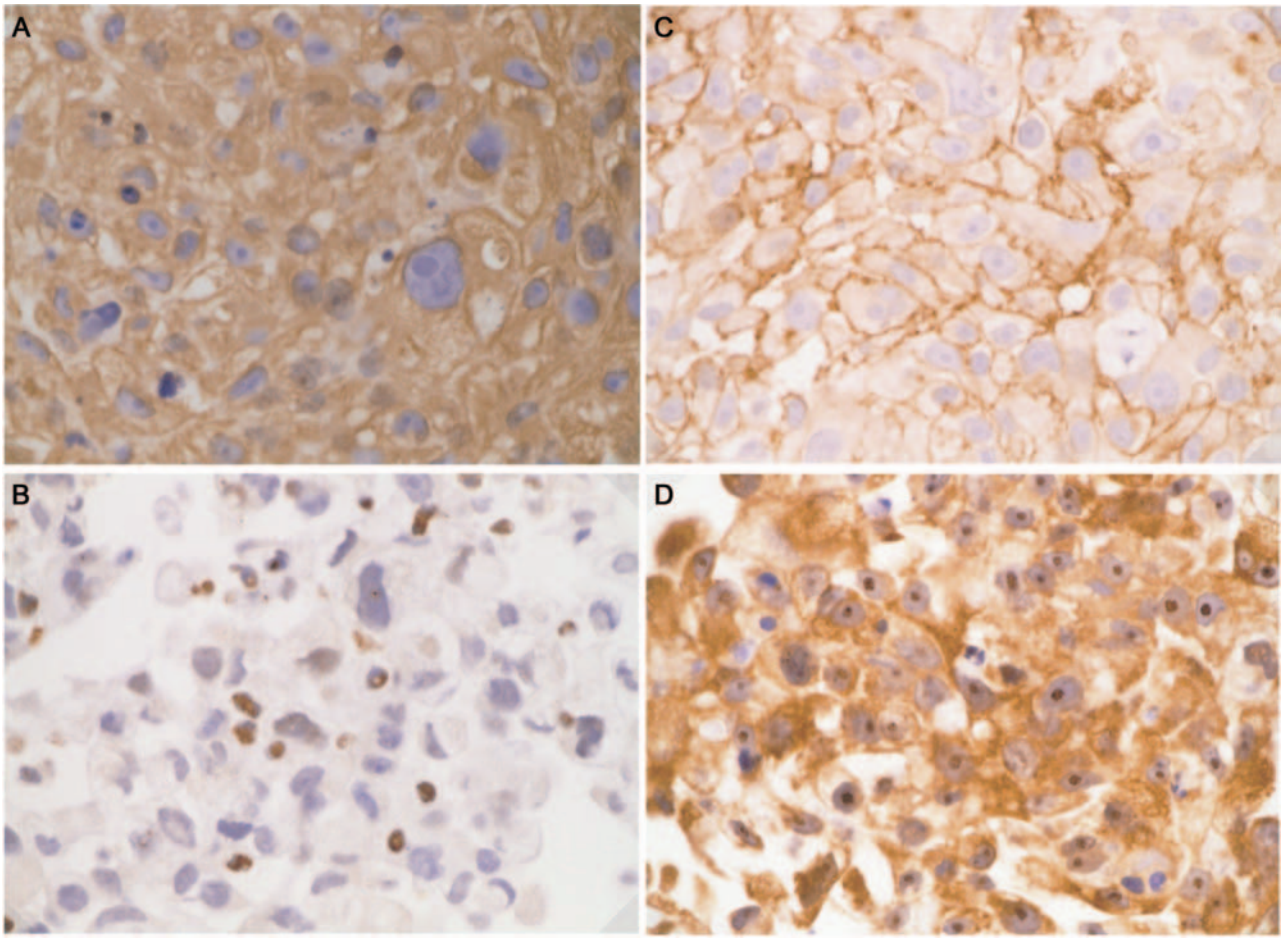

Figure 3. *FPS-1 cells immunohistochemically display positive immunoreactivity for vimentin (A), p53 (B), EGFR (C) and COX-2 (D).*

Establishment of the cell line. A new human UPS cell line, designated FPS-1, was established from the right upper arm tumor of the patient with UPS. The doubling time of the cell line was approximately 36.5 h. The cell line has been stably maintained for over 60 passages (a period of more than 14 months). The cultured cells consisted of spindle-shaped and pleomorphic cells associated with a few bizarre uninucleated giant cells (Figure 2). Loss of contact inhibition was observed. The cultured cells produced a large amount of mucinous material in the culture medium. Immunohistochemically, the cultured cells also reacted positively to vimentin (Figure 3A), CD68, CD99, lysozyme, p53 (approximately 7.4% positive cells, Figure 3B), Ki-67, EGFR (Figure 3C), and COX-2 (Figure 3D), but were negative for desmin, α-SMA, caldesmon, S-100 protein, AE1/AE3, CD34 and AT (Table I).

Heterotransplantation. The FPS-1 cell line showed successful heterotransplantation into SCID mice and athymic nude mice. In 2 out of 2 SCID mice and 2 out of 2 nude mice given a single subcutaneous injection of FPS-1 cells, a tumor measuring 7 x 5 x 5 mm developed 10 weeks after inoculation (Figure 4A). The cut surface of the xenografted tumors was solid and white with mucus. Light-microscopically, the tumor showed various morphological patterns: spindle-shaped or pleomorphic cells arranged in a storiform pattern with scattered uninucleated anaplastic cells (Figure 4B). A myxomatous area occupied most of the tumor tissue (Figure 4C). There were no metastases to other sites, such as the brain, lung or liver. Immunohistochemical study showed that the primary tumor cells had an immunoprofile similar to that of FPS-1 cells *in vitro* (Table I). However, the p53 gene product protein showed negative immunoreactivity.

Cytogenetics. Karyotype analysis of G-banded chromosomes for FPS-1 cells revealed the following composite karyotype: 61-69<3n>, XX, -Y, add(X)(p11), -2, -4, +5, +5, der(5)t(5;15)(q11;q11)x2, i(5)(p10), +7, +7, add(7)(q22), +8, +9, i(11)(q10), -13, -14, -15, -15, der(15)t(5;15)(q14;q22), der(15)t(5;15), +16, -17, -18, +20, -21, -21, -22, +0-4mar (Figure 5).
PCR-SSCP and direct sequencing analysis for p53 gene. In the

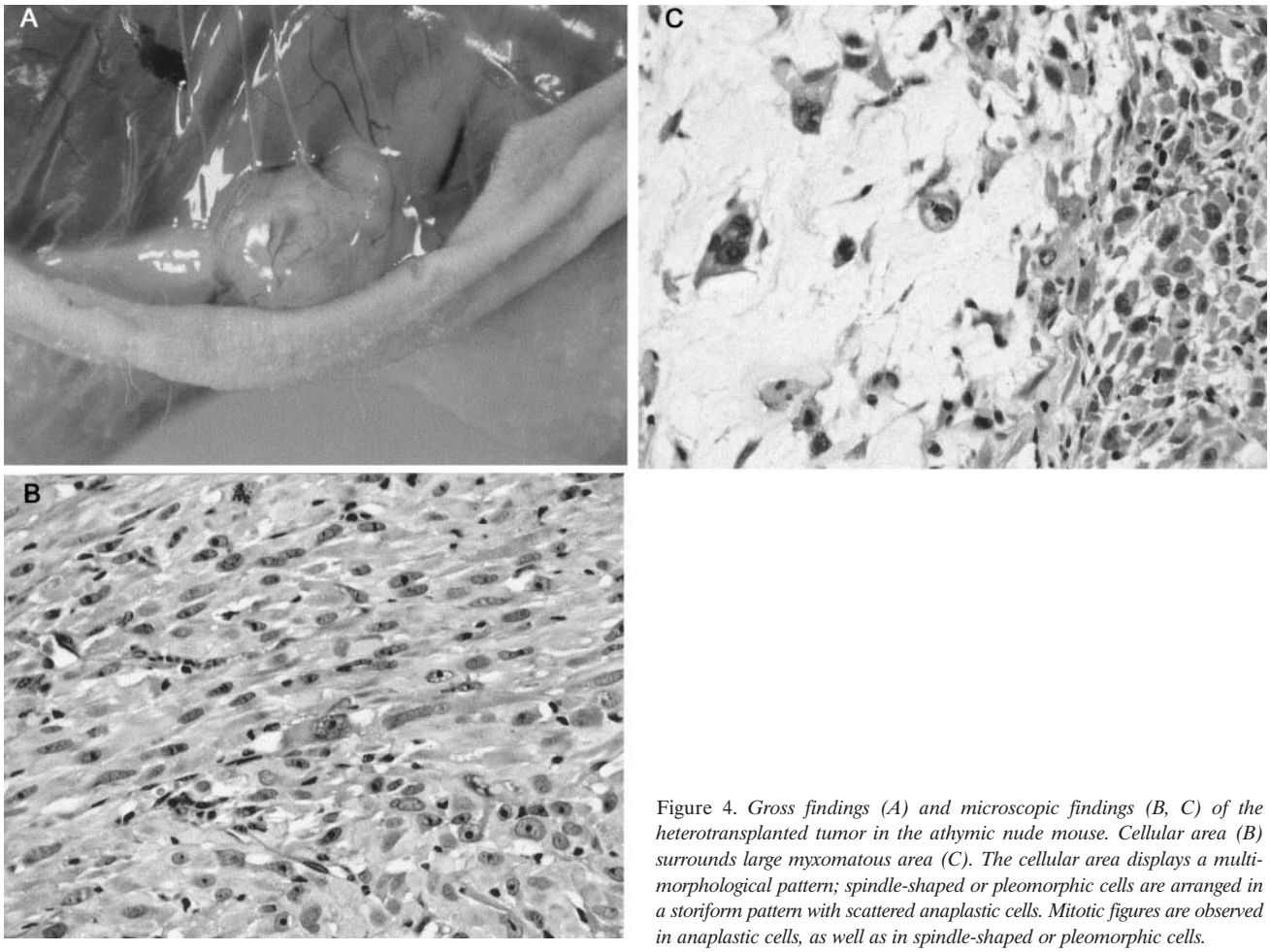

Figure 4. *Gross findings (A) and microscopic findings (B, C) of the heterotransplanted tumor in the athymic nude mouse. Cellular area (B) surrounds large myxomatous area (C). The cellular area displays a multimorphological pattern; spindle-shaped or pleomorphic cells are arranged in a storiform pattern with scattered anaplastic cells. Mitotic figures are observed in anaplastic cells, as well as in spindle-shaped or pleomorphic cells.*

FPS-1 cell line, an abnormal peak was detected in exon 6 of the p53 gene using PCR-SSCP (Figure 6A). The direct sequencing method detected a point mutation of the p53 gene from CGA to TGA at codon 213, which resulted in the conversion from arginine to a stop codon (nonsense mutation) in exon 6 (Figure 6B). No other abnormalities were detected.

Western blotting for EGFR and COX-2 proteins. Western blot analysis demonstrated that FPS-1 cells and FRTK-1 positive control cells expressed both EGFR and COX-2 proteins (Figure 7).

RT-PCR analysis for EGFR and COX-2 mRNA. RT-PCR analysis showed both EGFR and COX-2 mRNA in FPS-1 cells and in FRTK-1 positive control cells (Figure 8).

Discussion

We established the FPS-1 cell line derived from human UPS. FPS-1 cells showed various morphological characteristics: spindle-shaped or pleomorphic cells with atypical nuclei arranged in a storiform pattern with scattered uninucleated anaplastic cells. A myxomatous component was observed in the heterotransplanted tumor but not in the primary tumor. The immunophenotype of FPS-1 cells *in vitro* and *in vivo* was similar to that of the primary tumor cells, and FPS-1 was thought to be derived from the primary UPS.

Mutations in the p53 gene are the most common genetic changes found in human cancer and have been associated with poor prognosis in a large number of neoplasms. Previous studies of the p53 gene in UPS, identified point mutations in approximately 30% of cases, but no specific site of presentation or 'hot spot' has been reported. Until now, a p53 gene mutation has been detected in only one UPS cell line: GBS-1 [a missense mutation (Phe/Leu) due to transition (TTC/TTA) in codon 312 of exon 7] (20). In the current study, a p53 gene mutation was detected in the FPS-1 cell line: a nonsense mutation (Arg/Stop) due to transition (CGA/TGA) in codon 213 of exon 6. The previously reported p53 gene mutations of UPS cases in exon 6 are as follows: a transition (CGA/TGA) concerning codon 196

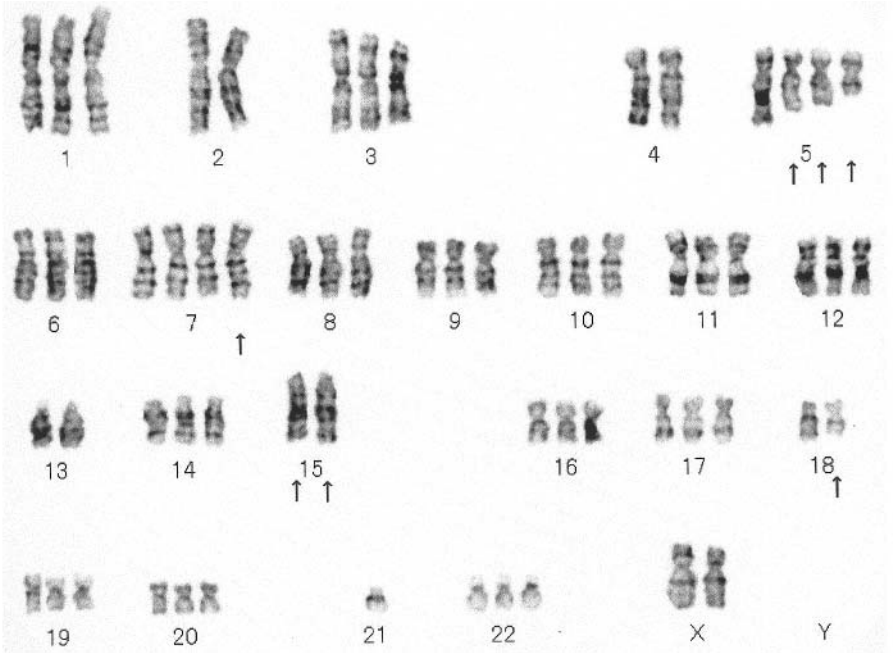

Figure 5. *The representative G-banded karyotype of the FPS-1 cell line at passage 39 exhibiting the following abnormal complement: 63, XX, -Y, -4, +5, der(5)t(5;15)(q11;q11)x2, -13, -15, -18, -21 (arrows).*

(Arg/Stop) (28), a transversion (CAT/GTT) concerning codon 193 (His/Val) (29), a transition (CGA/CGG) concerning codon 213 (Arg/Arg) and a 63-bp in-frame duplication, affecting codons 199-219 (30). The p53 gene mutation detected here in FPS-1 has not previously been reported in sarcomas. Several cases or cell lines with a missense mutation in the p53 gene were reported to show strong immunoreactivity to the p53 gene product protein. On the other hand, several cases or cell lines with a nonsense mutation showed undetectable or low levels of p53 gene product protein (31-33). The absence or lower levels of immunopositivity for the p53 gene product protein of FPS-1 cells both *in vitro* and *in vivo* (heterotransplanted tumor) was attributable to the clone harboring a nonsense mutation of the p53 gene.

EGFR is a receptor tyrosine kinase and is widely expressed in a variety of epithelial malignancies (34). EGFR activation promotes tumor growth by increasing cell proliferation, motility or angiogenesis, and by blocking apoptosis (35). Recently, an EGFR tyrosine kinase inhibitor, ZD1839 (Gefitinib, Iressa®), has been used clinically for lung cancer, and its antitumor effects on several types of malignancies have been investigated. The expression of EGFR and its ligands in sarcomas, including UPS, has been reported previously (36-40). Even though EGFR is recognized, from the standpoint of molecular targeting therapy, as a new target for UPS therapy, there has been no report on the effectiveness of ZD1839 for UPS.

COX, also known as prostaglandin H2 synthase or prostaglandin endoperoxide synthase, is the key enzyme in the conversion of arachidonic acid to prostanoids (41). Two COX genes have been cloned: COX-1 is a constitutive enzyme produced constantly in most tissue types and is probably responsible for the production of prostanoids under physiological conditions. COX-2 is undetectable in most normal tissues, but can be induced in various cell types by pro-inflammatory agents, growth factors and carcinogens (42). Overexpression of COX-2 has been found in a various malignancies, including carcinomas, lymphomas and some sarcomas. In addition, COX-2 activation promotes tumor growth through the production of prostaglandin E2, which could block apoptosis or activate vascular endothelial growth factor and angiogenesis (43). Recent studies have shown antitumor effects of some selective COX-2 inhibitors for sarcomas (44) as well as for carcinomas. COX-2 expression in UPS was found immunohistochemically in tumor specimens (45, 46) and selective COX-2 inhibitor inhibited cell growth *in vitro* in two UPS cell lines (47). Therefore, COX-2 is now recognized as a new target for UPS therapy.

FPS-1 cells expressed both EGFR and COX-2. Recently, crosstalk between EGFR and COX-2 has been found (48, 49) and cooperative cell-growth inhibition by the combination treatment with EGFR tyrosine kinase inhibitor and a selective COX-2 inhibitor in some carcinomas was reported (50-53). However, there has been no report on a combined antitumor effect of an EGFR tyrosine kinase inhibitor and a selective COX-2 inhibitor in sarcoma. Thus, the FPS-1 cell line is useful

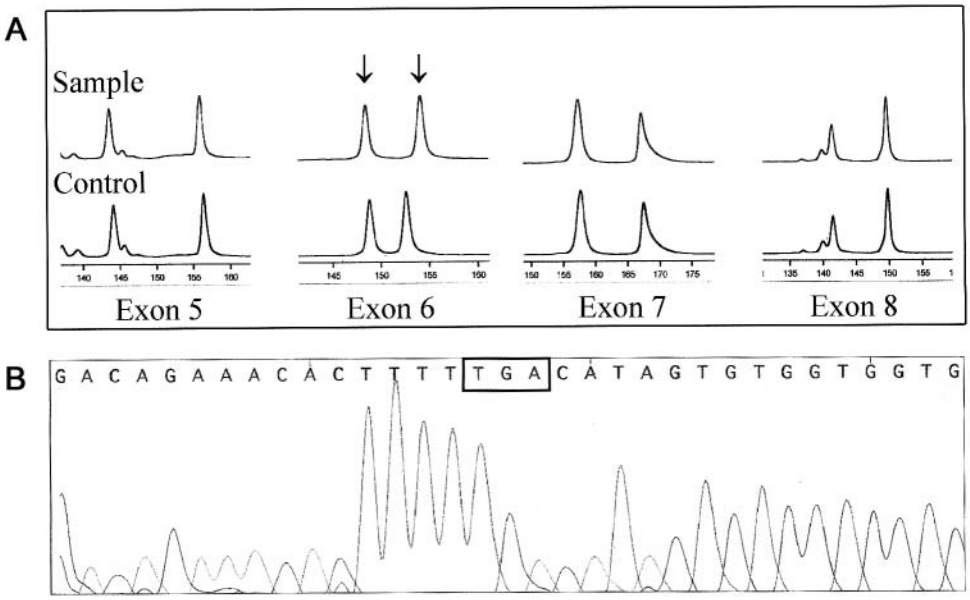

Figure 6. *PCR-SSCP analysis (A) and direct sequencing analysis (B) of the p53 gene. PCR-SSCP analysis exhibits abnormal peaks in exon 6 (arrows). Direct sequence shows a nonsense mutation in exon 6 from CGA (arginine) to TGA (stop codon) at codon 213.*

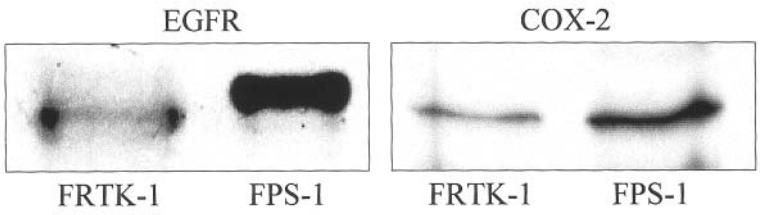

Figure 7. *Western blot analyses of EGFR (170 kDα) and COX-2 (70 kDα) protein of FPS-1 and FRTK-1 positive control cell lines. The bands of membranous protein EGFR and cytoplasmic protein COX-2 are detected.*

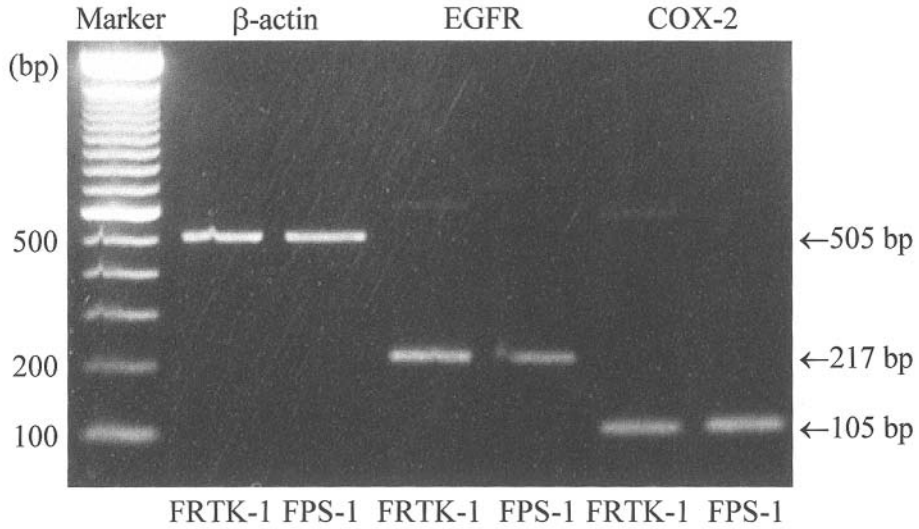

Figure 8. *RT-PCR analysis of EGFR and COX-2 mRNA in FPS-1 and FRTK-1 cell lines. Lane 1, Marker, 100-bp DNA ladder; Lanes 2 and 3, β-actin (505 bp); Lanes 4 and 5, EGFR (217 bp); Lanes 6 and 7, COX-2 (105 bp). EGFR and COX-2 mRNA are detected.*

for investigating the antitumor effect of EGFR tyrosine kinase inhibitor combined with selective COX-2 inhibitors.

In conclusion, we established a new cell line (FPS-1) derived from UPS (MFH). The FPS-1 cells showed the same morphological characteristics and immunophenotype as those of the primary tumor cells that are characteristic of UPS; in addition they had a nonsense mutation of the p53 gene in exon 6 as well as expression of EGFR and COX-2. The FPS-1 cell line is useful for investigating biological behavior and developing UPS antitumor therapies.

Acknowledgements

The authors thank Mrs Hiromi Kaneko and Ms Satomi Hikichi for their skillful technical assistance.

References

1 Fletcher CDM, van den Berg E and Molenaar WM: Pleomorphic malignant fibrous histiocytoma / undifferentiated high grade pleomorphic sarcoma. *In*: World Health Organization Classification of Tumours; Tumours of Soft Tissue and Bone. Fletcher CDM, Unni KK and Mertens F (eds.). Lyon, IARC Press, pp. 120-122, 2002.

2 Weiss SW and Goldblum JR: Malignant fibrohistiocytic tumors. *In*: Enzinger and Weiss's Soft Tissue Tumors. 4th edition. Weiss SW and Goldblum JR (eds.). St. Louis, Mosby, pp. 535-569, 2001.

3 Hecht F, Berger C, Hecht BK and Speiser BL: Fibrous histiocytoma cell line: chromosome studies of mouse and man. Cancer Genet Cytogenet *8*: 359-362, 1983.

4 Shirasuna K, Sugiyama M and Miyazaki T: Establishment and characterization of neoplastic cells from a malignant fibrous histiocytoma. A possible stem cell line. Cancer *55*: 2521-2532, 1985.

5 Roholl PJ, Kleyne J, Van Blokland M, Spies PL, Rutgers DH, Albus-Lutter CE and Van Unnik JA: Characterization of two cell lines, derived from two malignant fibrous histiocytomas. J Pathol *150*: 103-112, 1986.

6 Iwasaki H, Isayama T, Ohjimi Y, Kikuchi M, Yoh S, Shinohara N, Yoshitake K, Ishiguro M, Kamada N and Enjoji M: Malignant fibrous histiocytoma. A tumor of facultative histiocytes showing mesenchymal differentiation in cultured cell lines. Cancer *69*: 437-447, 1992.

7 Takahashi K and Sato K: Establishment and characterization of a human neoplastic cell line (MFH-ino) derived from malignant fibrous histiocytoma of maxilla. Hum Cell *4*: 51-57, 1991.

8 Kanzaki T, Kitajima S and Suzumori K: Biological behavior of cloned cells of human malignant fibrous histiocytoma *in vivo* and *in vitro*. Cancer Res *51*: 2133-2137, 1991.

9 Roholl PJ, Prinsen I, Rademakers LP, Hsu SM and Van Unnik JA: Two cell lines with epithelial cell-like characteristics established from malignant fibrous histiocytomas. Cancer *68*: 1963-1972, 1991.

10 Sekiguchi M, Asanuma K, Shiroko Y, Mori S, Shimoda T and Kamada N: Biological characteristics of a continuous cultured cell line derived from a human malignant fibrous histiocytoma. Hum Cell *7*: 199-206, 1994.

11 Yonemoto T, Takenouchi T, Tokita H, Tatezaki S, Mukaida N, Mikata A and Moriya H: Establishment and characterization of a human malignant fibrous histiocytoma cell line. Clin Orthop Relat Res *320*: 159-167, 1995.

12 Yokota T, Ishizaki R, Ohishi H and Ohami H: Establishment and characterization of a fibrous histiocytoma cell line (NMSG 10) derived from a malignant fibrous histiocytoma. Pathol Res Pract *191*: 1231-1238, 1995.

13 Krause AK, Hinrichs SH, Orndal C, DeBoer J, Neff JR and Bridge JA: Characterization of a human myxoid malignant fibrous histiocytoma cell line, OH931. Cancer Genet Cytogenet *94*: 138-143, 1997.

14 Reinecke P, Moll R, Hildebrandt B, Schmitz M, Schneider EM, Koldovsky P, Schardt C, Gabbert HE and Gerharz C: A novel human malignant fibrous histiocytoma cell line of the heart (MFH-H) with secretion of hematopoietic growth factors. Anticancer Res *19*: 1901-1907, 1999.

15 Endo K, Sakatani T, Watanabe M, Yoshida H, Nanba E and Ito H: Wild-type p53 gene transfer resulted in cell cycle arrest, but not apoptosis of newly established human malignant fibrous histiocytoma cell line. Int J Oncol *15*: 935-942, 1999.

16 Mairal A, Chibon F, Rousselet A, Couturier J, Terrier P and Aurias A: Establishment of a human malignant fibrous histiocytoma cell line, COMA. Characterization by conventional cytogenetics, comparative genomic hybridization, and multiplex fluorescence *in situ* hybridization. Cancer Genet Cytogenet *121*: 117-123, 2000.

17 Kiyozuka Y, Nakagawa H, Uemura Y, Senzaki H, Yamamoto A, Noguchi T, Mizuta H, Nakanishi K, Nakano S and Tsubura A: Novel cell lines established from a human myxoid malignant fibrous histiocytoma arising in the uterus. Cancer Genet Cytogenet *127*: 7-15, 2001.

18 Mori A, Tagawa T, Kamei T, Murata T, Inui M and Ohse S: Characterization of four cell lines derived from a human malignant fibrous histiocytoma of the maxillary sinus. Oral Oncol *37*: 527-536, 2001.

19 Nakatani T, Marui T, Yamamoto T, Kurosaka M, Akisue T and Matsumoto K: Establishment and characterization of cell line TNMY1 derived from human malignant fibrous histiocytoma. Pathol Int *51*: 595-602, 2001.

20 Fang Z, Mukai H, Nomura K, Shinomiya K, Matsumoto S, Kawaguchi N, Kitagawa T and Kanda H: Establishment and characterization of a cell line from a malignant fibrous histiocytoma of bone developing in a patient with multiple fibrous dysplasia. J Cancer Res Clin Oncol *128*: 45-49, 2002.

21 Nishio J, Iwasaki H, Ishiguro M, Ohjimi Y, Nishimura N, Koga T, Kawarabayashi T, Kaneko Y and Kikuchi M: Establishment of a new human malignant fibrous histiocytoma cell line, FU-MFH-1: cytogenetic characterization by comparative genomic hybridization and fluorescence *in situ* hybridization. Cancer Genet Cytogenet *144*: 44-51, 2003.

22 Murakami Y, Hayashi K and Sekiya T: Detection of aberrations of the *p53* alleles and the gene transcript in human tumor cell lines by single-strand conformation polymorphism analysis. Cancer Res *51*: 3356-3361, 1991.

23 Kuze T, Nakamura N, Hashimoto Y, Hojo H, Abe M and Wakasa H: The relationship between the gene mutation of p53 and the protein expression of p53 and Ki-67 in non-Hodgkin's lymphomas. Pathol Int *46*: 130-136, 1996.

24 Hashimoto Y, Nakamura N, Kuze T, Abe M and Wakasa H: Intranuclear expression of cyclin D1 protein as a useful prognostic marker for mantle cell lymphoma. Fukushima J Med Sci *43*: 87-98, 1997.

25 Narita T, Taga T, Sugita K, Nakazawa S and Ohta S: The autocrine loop of epidermal growth factor receptor-epidermal

growth factor / transforming growth factor-alpha in malignant rhabdoid tumor cell lines: heterogeneity of autocrine mechanism in TTC549. Jpn J Cancer Res *92*: 269-278, 2001.
26 Yuan A, Yu CJ, Shun CT, Luh KT, Kuo SH, Lee YC and Yang PC: Total cyclooxygenase-2 mRNA levels correlate with vascular endothelial growth factor mRNA levels, tumor angiogenesis and prognosis in non-small cell lung cancer patients. Int J Cancer *115*: 545-555, 2005.
27 Hakozaki M, Hojo H, Sato M, Kaneko Y, Watanabe N, Kikuchi S and Abe M: Establishment and characterization of a new cell line, FRTK-1, derived from human malignant rhabdoid tumor of the kidney, with over expression of epidermal growth factor receptor and cyclooxygenase-2. Oncol Rep *16*: 265-271, 2006.
28 Toguchida J, Yamaguchi T, Ritchie B, Beauchamp RL, Dayton SH, Herrera GE, Yamamuro T, Kotoura Y, Sasaki MS, Little JB, Weichselbaum RR, Ishizaki K and Yandell DW: Mutation spectrum of the p53 gene in bone and soft tissue sarcomas. Cancer Res *52*: 6194-6199, 1992.
29 Yoo J, Lee HK, Kang CS, Park WS, Lee JY and Shim SI: p53 gene mutations and p53 protein expression in human soft tissue sarcomas. Arch Pathol Lab Med *121*: 395-399, 1997.
30 Molina P, Pellin A, Navarro S, Boix J, Carda C and Llombart-Bosch A: Analysis of p53 and mdm2 proteins in malignant fibrous histiocytoma in absence of gene alteration: prognostic significance. Virchows Arch *435*: 596-605, 1999.
31 Davidoff AM, Pence JC, Shorter NA, Iglehart JD and Marks JR: Expression of p53 in human neuroblastoma- and neuroepithelioma-derived cell lines. Oncogene *7*: 127-133, 1992.
32 Villuendas R, Piris MA, Algara P, Sanchez-Beato M, Sanchez-Verde L, Martinez JC, Orradre JL, Garcia P, Lopez C and Martinez P: The expression of p53 protein in non-Hodgkin's lymphomas is not always dependent on p53 gene mutations. Blood *82*: 3151-3156, 1993.
33 Lepelley P, Preudhomme C, Vanrumbeke M, Quesnel B, Cosson A and Fenaux P: Detection of p53 mutations in hematological malignancies: comparison between immuno-cytochemistry and DNA analysis. Leukemia *8*: 1342-1349, 1994.
34 Nicholson RI, Gee JM and Harper ME: EGFR and cancer prognosis. Eur J Cancer *37*: S9-15, 2001.
35 Holbro T, Civenni G and Hynes NE: The ErbB receptors and their role in cancer progression. Exp Cell Res *284*: 99-110, 2003.
36 Dobashi Y, Takei N, Suzuki S, Yoneyama H, Hanawa M and Ooi A: Aberration of epidermal growth factor receptor expression in bone and soft-tissue tumors: protein overexpression, gene amplification and activation of downstream molecules. Mod Pathol *17*: 1497-1505, 2004.
37 Yamamoto T, Fujita I, Akisue T, Marui T, Nakatani T, Kawamoto T, Hitora T, Nagira K, Matsumoto K and Kurosaka M: Amphiregulin and epidermal growth factor receptor expression in human malignant fibrous histiocytoma of soft tissues. Anticancer Res *24*: 1307-1310, 2004.
38 Yamamoto T, Akisue T, Marui T, Nakatani T, Kawamoto T, Hitora T, Nagira K, Fujita I, Matsumoto K and Kurosaka M: Expression of betacellulin, heparin-binding epidermal growth factor and epiregulin in human malignant fibrous histiocytoma. Anticancer Res *24*: 2007-2010, 2004.
39 Beech D, Pollock RE, Tsan R and Radinsky R: Epidermal growth factor receptor and insulin-like growth factor-I receptor expression and function in human soft-tissue sarcoma cells. Int J Oncol *12*: 329-336, 1998.
40 Abdiu A, Walz TM, Nishikawa BK, Wingren S, Larsson SE and Wasteson A: Human malignant fibrous histiocytomas *in vitro*: growth characteristics and their association with expression of mRNA for platelet-derived growth factor, transforming growth factor-alpha and their receptors. Eur J Cancer *34*: 2094-2100, 1998.
41 Vane JR, Bakhle YS and Botting RM: Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol *38*: 97-120, 1998.
42 Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB and Lipsky PE: Cyclooxygenase in biology and disease. FASEB J *12*: 1063-1073, 1998.
43 Gately S and Li WW: Multiple roles of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy. Semin Oncol *31*: 2-11, 2004.
44 Dickens DS and Cripe TP: Effect of combined cyclooxygenase-2 and matrix metalloproteinase inhibition on human sarcoma xenografts. J Pediatr Hematol Oncol *25*: 709-714, 2003.
45 Yamashita H, Osaki M, Ardyanto TD, Osaki M, Yoshida H and Ito H: Cyclooxygenase-2 in human malignant fibrous histiocytoma: correlations with intratumoral microvessel density, expression of vascular endothelial growth factor and thymidine phosphorylase. Int J Mol Med *14*: 565-570, 2004.
46 Lassus P, Ristimaki A, Huuhtanen R, Tukiainen E, Asko-Seljavaara S, Andersson LC, Miettinen M, Blomqvist C, Haglund C and Bohling T: Cyclooxygenase-2 expression in human soft-tissue sarcomas is related to epithelial differentiation. Anticancer Res *25*: 2669-2674, 2005.
47 Yamashita H, Osaki M, Honjo S, Yoshida H, Teshima R and Ito H: A selective cyclooxygenase-2 inhibitor, NS-398, inhibits cell growth by cell cycle arrest in a human malignant fibrous histiocytoma cell line. Anticancer Res *23*: 4671-4676, 2003.
48 Dannenberg AJ, Lippman SM, Mann JR, Subbaramaiah K and DuBois RN: Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. J Clin Oncol *23*: 254-266, 2005.
49 Lippman SM, Gibson N, Subbaramaiah K and Dannenberg AJ: Combined targeting of the epidermal growth factor receptor and cyclooxygenase-2 pathways. Clin Cancer Res *11*: 6097-6099, 2005.
50 Tortora G, Caputo R, Damiano V, Melisi D, Bianco R, Fontanini G, Veneziani BM, De Placido S, Bianco AR and Ciardiello F: Combination of a selective cyclooxygenase-2 inhibitor with epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 and protein kinase A antisense causes cooperative antitumor and antiangiogenic effect. Clin Cancer Res *9*: 1566-1572, 2003.
51 Chen Z, Zhang X, Li M, Wang Z, Wieand HS, Grandis JR and Shin DM: Simultaneously targeting epidermal growth factor receptor tyrosine kinase and cyclooxygenase-2, an efficient approach to inhibition of squamous cell carcinoma of the head and neck. Clin Cancer Res *10*: 5930-5939, 2004.
52 Zhang X, Chen ZG, Choe MS, Lin Y, Sun SY, Wieand HS, Shin HJ, Chen A, Khuri FR and Shin DM: Tumor growth inhibition by simultaneously blocking epidermal growth factor receptor and cyclooxygenase-2 in a xenograft model. Clin Cancer Res *11*: 6261-6269, 2005.
53 Melisi D, Caputo R, Damiano V, Bianco R, Veneziani BM, Bianco AR, De Placido S, Ciardiello F and Tortora G: Zoledronic acid cooperates with a cyclooxygenase-2 inhibitor and gefitinib in inhibiting breast and prostate cancer. Endocr Relat Cancer *12*: 1051-1058, 2005.

Received May 23, 2006
Accepted July 12, 2006