Abstract. Background: FUS1 is a tumor suppressor gene located on human chromosome 3p21.3. Frequent loss of FUS1 protein expression is associated with lung cancer development. This study examined FUS1 expression and its possible tumor-suppressive role in bone and soft tissue sarcomas. Materials and Methods: The expressions of FUS1 mRNA and FUS1 protein were assessed in sarcoma cell lines, sarcoma tissues, benign bone and soft-tissue tumor (BST) tissues, and healthy tissues. Exogenous FUS1 gene transfection was performed on sarcoma cell lines. Results: FUS1 mRNA expression was detected in all sarcoma cell lines, all benign BSTs and healthy tissues, and almost all sarcoma tissues. In contrast, FUS1 protein expression was frequently lost in sarcoma cells and sarcoma tissues. The exogenous FUS1 gene delivery induced strong FUS1 protein expression, inhibition of cell viability and apoptosis in sarcoma cells. Conclusion: FUS1 may act as a tumor suppressor in bone and soft-tissue sarcomas.

Bone sarcoma and soft-tissue sarcoma account for fewer than 0.2% and 1% of all human carcinomas, respectively, but they can be life threatening (1). Despite significant advances in multidisciplinary treatment, the long-term survival rates for advanced bone and soft tissue sarcomas are poor (2-4).

Molecular genetic studies, such as identification of novel oncogenes and tumor suppressor genes, will greatly contribute to understanding the pathogenesis and progression of sarcomas, and, thus, will promote the development of molecular-targeted therapy for various types of sarcomas.

FUS1 is a novel tumor suppressor gene that was identified in a 120-kb crucial region on human chromosome 3p21.3, where homozygous deletions frequently occur in lung and breast cancer (5-8). The FUS1 gene product is a multi-functional protein and plays an important role in various cellular processes, including transcription, signal transduction, cell-cycle progression and apoptosis (8). High-level FUS1 mRNA expression has been detected in various healthy human tissues such as heart, brain, placenta and lung, using multiple-tissue Northern blots (5). Unlike other tumor suppressor genes, mutation of the FUS1 gene is infrequent in the majority of lung carcinomas (5). In contrast, expression of the FUS1 protein is frequently lost or reduced in primary lung cancer and is associated with lung cancer development and a worse survival rate (9). Overexpression of wild-type FUS1 in FUS1 protein-deficient tumor cells dramatically suppressed tumor cell growth and induced apoptosis in vitro and in vivo (6-8). Systemic treatment with a combination of FUS1 nanoparticles and p53 or cisplatin in a human lung-cancer xenograft mouse model synergistically suppressed tumor growth and induced apoptosis (10, 11).

To the Authors’ knowledge, there have been no reports regarding FUS1 expression in human bone and soft tissue sarcomas. To clarify whether the tumor suppressor FUS1 also acts as a tumor suppressor gene in bone and soft-tissue sarcomas, this study analyzed FUS1 gene and protein expression in various types of healthy and sarcoma cell lines and tissue samples, including a series of bone and soft tissue sarcomas, benign bone and soft tissue tumors (BSTs) and...
healthy bone and soft tissues. Moreover, the study determined whether forced exogenous FUS1 gene expression may inhibit the tumor growth of sarcoma cells in vitro.

Materials and Methods

Cell lines and cell culture. Sarcoma cell lines, including the osteosarcoma cell lines NOS1 (12) and NOS10 (13), the malignant fibrous histiocytoma (MFH) cell lines NMFH1(14) and NMFH2 (15), the malignant peripheral nerve sheath tumor cell line NMS-2 (16), and a normal human bone marrow fibroblast (BMF) cell line, were established under the approval of the Institutional Review Board (Niigata University Hospital). The alveolar soft part sarcoma cell line ASPS-KY was a gift from Dr. S. Yanoma (Kanagawa Cancer Center, Yokohama, Japan); the synovial sarcoma HS-SY-II cell line (17) was a gift from Dr. H. Sonobe (Department of Pathology, Kochi Medical School, Kochi, Japan); the epithelioid sarcoma cell line SFT8606 (18) was a gift from Dr. H. Iwasaki (Fukuoka University School of Medicine, Fukuoka, Japan); and the liposarcoma cell line 402-92 (19) was a gift from Dr. P. Åman (Department of Clinical Genetics, University Hospital, Lund, Sweden). The remaining cell lines used in this study were obtained from commercial sources (Table I).

Tissue samples. A total of 147 malignant and benign BST tissue samples (Table II) were obtained from 135 patients who had received surgical resection or open biopsy (79 males and 56 females) from 2001 to 2008 in Niigata University Hospital, Japan. These BST samples included 95 non-metastatic sarcomas, 12 metastatic sarcomas and 12 distant metastatic foci, as well as 28 benign BSTs (Table II). After resection, each tumor sample was divided into two pieces: one was stored at –80˚C, and the other was formalin-fixed, paraffin-embedded and, then, diagnosed by experienced pathologists according to the WHO classification system (20). Healthy tissues including tissues from the skin, bone, muscle and fat (three each), peripheral nerve and blood vessels (two each) and tendon and fascia (one each) were obtained from patients suffering from curative amputation or other non-tumor diseases and served as healthy controls. This study was approved by the Institutional Review Board of Niigata University Hospital and informed consent was obtained from all patients.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Total RNA was extracted from cell lines (Table I), frozen tumor tissue samples (Table II) and healthy bone and soft tissues using...
Isogen Reagent (Nippongene, Toyama, Japan) according to the manufacturer's instructions. Total RNA (1.0 μg) was converted to cDNA using M-MLV RT (Invitrogen) and oligo dT primers (Promega, Madison, WI, USA). An equal amount of cDNA (1 μl) was amplified by PCR using \(FUS_1\)-specific oligonucleotide primer pairs: forward, 5'-ATGATGAGGATGGGGATCTG-3'; reverse, 5'-GAGGATCACAGGGAAATCCA-3'. The PCR reaction conditions were as follows: 94˚C for 2 min, followed by 30 cycles of 94˚C for 30 s, 56˚C for 30 s, and 72˚C for 90 s, with a final extension at 72˚C for 5 min. A plasmid vector encoding \(FUS_1\) cDNA was used as a positive control. RT-PCR of the housekeeping gene \(GAPDH\) was used as an internal control and its amplification was performed as previously described (21).

**Immunohistochemistry using a tissue microarray.** Eighty-five formalin-fixed and paraffin-embedded tumor samples (Table III), as well as various healthy bone and soft tissue samples, were retrieved from the institutional archive (Niigata University Hospital). Prior to immunohistochemistry, the hematoxylin and eosin staining of all tumor samples was carefully examined under a microscope to select the appropriate area for further analysis. Three cylindrical cores (2-mm in diameter) were punched out from the areas of interest in each donor paraffin block and were re-embedded in recipient tissue microarray blocks, which were manually prepared. Immunohistochemical staining of \(FUS_1\) protein was performed using a rabbit anti-\(FUS_1\) polyclonal antibody that was raised against a synthetic oligopeptide (GASGSKARGLWPFASAA), derived from the NH2-terminal amino sequence of the \(FUS_1\) protein (Bethyl Laboratories, Montgomery, TX, USA) (8) and a previously reported protocol (9). Briefly, tissue microarray slides (4 μm) were deparaffinized and hydrated and antigen was retrieved by heating in 10 mM sodium citrate buffer (pH 6.0) using a steamer for 10 min at 98˚C. The slides were then blocked with 3% hydrogen peroxide in methanol at room temperature for 20 min, followed by 10% bovine serum albumin in Tris-buffered saline Tween-20 for 30 min. After incubation with the anti-\(FUS_1\) antibodies (1:400) for 60 min at room temperature, the slides were washed with PBS and stained using a Histofine Simple Stain PO (MULTI) kit (Nichirei, Tokyo, Japan) according to the manufacturer's instructions. The signals were then developed using DAB substrate (Nichirei) and, finally, the slides were counterstained with hematoxylin. Human healthy lung bronchial epithelia were used as a positive control.

**Transient transfection.** A recombinant plasmid vector encoding the wild-type \(FUS_1\) gene (wt-\(FUS_1\)) was used for transient exogenous gene transfection. A plasmid vector encoding the \(\beta\)-galactosidase gene (\(LacZ\)) was used as a nonspecific negative control. Construction of these plasmid vectors has been described previously (6, 8). Cultured cells were transfected with plasmid DNA using
FuGENE 6 Transfection Reagent (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer’s instructions. A FuGENE 6 reagent:DNA ratio of 3:1 (μg:μg) respectively was applied to each 60 mm culture dish or each well of a 96-well plate.

**Western blot analysis.** Cultured cells (Table I) were harvested and subjected to Western blot analysis to determine endogenous expression of the FUS1 protein. WI-38, a normal human lung fibroblast that is known to express endogenous FUS1 protein was used as a positive control (8). Eight sarcoma cell lines as well as the normal cell line WI-38 were chosen for exogenous FUS1 gene transfection. The cells were plated on 60 mm culture dishes at a density of 2x10^5/dish 24 h prior to treatment and, subsequently, the cells in each dish were transfected with 2 μg of wt-FUS1 or LacZ plasmid DNA using the FuGENE 6 reagent. At 48, 72 and 96 h post transfection, the cells were subjected to Western blotting to assess the expression level of the FUS1 protein. Briefly, the cells were washed with ice-cold PBS and were lysed by adding sample buffer (62.5 mM Tris, pH 6.8; 2% SDS; 5% glycerol and 6 M urea) containing a complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA) to each dish. The cell lysates were centrifuged at 14,000 g for 5 min at 4˚C, the supernatants were collected and the protein concentration of the lysates was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). Dithiothreitol (1 M; Sigma-Aldrich, St. Louis, MO, USA) and bromophenol blue were then added to the lysates to a final concentration of 5% (v/v) each and the lysates were boiled for 5 min. Equal amounts of proteins (50 μg/lane) were separated on 15% SDSPAGE gels (Bio-Rad, Hercules, CA, USA) for 1 h and were electrotransferred onto Hybond ECL nitrocellulose membranes (GE Healthcare, Little Chalfont, Buckinghamshire, UK) for 2 h. The membranes were blocked in blocking buffer (5% milk in 1 mM Tris-buffered saline containing 0.2% Tween-20) for 1 h, and were then probed overnight at 4˚C with a rabbit anti-FUS1 polyclonal antibody (1:1000). The immunoblots were subsequently incubated with horseradish peroxidase-labeled anti-rabbit IgG (GE Healthcare) for 1 h at room temperature and were developed using ECL detection reagents (GE Healthcare). The blots were reprobed using a mouse anti-β-actin monoclonal antibody (1:3000, Sigma-Aldrich) to ensure uniform sample loading.

**Cell viability and morphology.** Eight sarcoma cell lines were plated on 96-well plates (2x10^3 cells/well) 24 h before gene transfection. The cells in each well were transfected with 0.05 μg wt-FUS1 plasmid DNA using 0.15 μl FuGENE 6. As a control, cells were transfected with LacZ plasmid vectors. Cell viability was assessed using the XTT (sodium 3’-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate) assay 24, 48, 72 and 96 h after gene transfection using the Cell Proliferation Kit-II (Roche Diagnostics) according to the manufacturer’s protocol. In addition, 2x10^5 cells were also plated on 60 mm culture dishes and were, subsequently, transfected with 2 μg of wt-FUS1 plasmid DNA. Cell morphology was observed daily using an Olympus phase-contrast microscope UWLCD 0.30 (IMT2, Olympus, Tokyo, Japan) and photomicrographs were taken, at a magnification of x40, 72 h after transfection.

**Apoptosis analysis.** To assess the induction of apoptosis after wt-FUS1 plasmid DNA transfection, the cleavage of caspase-3 and poly (ADP-ribose)-polymerase (PARP) were analyzed by Western blot analysis. Six sarcoma cell lines (NOS10, SaOS2, ASPS-KY, HS-SY-II, NMS-2 and OUMS-27) were transfected with wt-FUS1 or LacZ plasmid DNA. The expression levels of cleaved caspase-3 and cleaved PARP proteins were analyzed at 48 and 72 h after transfection using rabbit anti-caspase-3 (1:1000; Cell Signaling Technology, Beverly, MA, USA) and anti-PARP polyclonal antibodies (1:1000; Cell Signaling Technology), respectively. The Western blotting procedure was performed as described earlier except that the proteins were loaded on a 7.5% SDS-PAGE gel for analysis of the cleaved PARP protein.

**Statistical analysis.** All data were evaluated using SPSS 14.0 software (SPSS Inc., Chicago, IL, USA). Correlations between tumor histological types and FUS1 expression (mRNA and protein) were assessed using Fisher’s exact test. The level of statistical significance was set to p<0.05. The statistical significance of differences between wt-FUS1 and LacZ gene transfection groups were evaluated using a two-tailed paired sample t-test. Data are presented as mean±standard deviation. A p-value <0.01 denoted statistical significance.

**Results**

**Loss of FUS1 gene expression is rare in both sarcomas and benign BSTs.** FUS1 gene expression was analyzed in three human normal fibroblasts, 17 sarcoma cell lines (Table I), 119 sarcoma samples, 28 benign BSTs (Table II) and in various healthy bone and soft tissue samples using RT-PCR analysis. A FUS1 mRNA product, 164 bp in size, was detected in all normal and sarcoma cell lines (Figure 1A), in all benign BSTs (100%) and in 117 out of 119 (98%) sarcoma samples (Figure 1B and Table II), as well as in all healthy bone and soft tissue samples (Figure 1C). Complete loss of FUS1 mRNA expression was observed in only two cases, both of which were MFH tumor samples (Figure 1B). There were no significant differences in FUS1 mRNA expression between the sarcomas and the benign BSTs or between the non-metastatic and the metastasized sarcoma samples.

**FUS1 protein expression is frequently absent from sarcomas, benign BSTs and healthy bone and soft tissues.** FUS1 protein expression was analyzed in normal fibroblasts and in sarcoma cell lines (Table I) by Western blot analysis. Compared with its expression in normal lung fibroblast WI-38 cells (positive control), FUS1 protein expression was relatively low in two normal fibroblast cell lines of mesenchymal origin (BMF and NHDF), was extremely low in eight of the 17 sarcoma cell lines (HOS, MG-63, NOS1, OST, SaOS2, NMFH1, NMFH2 and SFT-8606) and was completely absent from 9 of the 17 sarcoma cell lines (HuO9, NOS10, U2-OS, ASPS-KY, HS-SY-II, NMS-2, OUMS-27, SKNMC and 402-92) (Figure 1D). FUS1 protein expression was also analyzed by immunohistochemical staining of a large number of malignant and benign BSTs and of various healthy bone and soft tissues. None of the 70
Figure 1. FUS1 expression in human sarcoma cell lines, BST samples and in normal bone and soft tissues. The expression of FUS1 mRNA was determined in 3 human normal fibroblasts and 17 sarcoma cell lines (A); representative cases of sarcomas and benign BSTs (OS1-5, osteosarcoma; MFH1-11, malignant fibrous histiocytoma; Ch1-5, chondrosarcoma; GCT1-5, giant cell tumor of bone; Schw1-5, Schwannoma) (B); and normal bone and soft tissues (C), using RT-PCR analysis and the FUS1 gene-specific primer sets described in ‘Materials and Methods’. A FUS1-expressing plasmid cDNA was used as a positive control and GAPDH mRNA was used as an internal control. Western blot analysis of FUS1 protein expression in human normal fibroblasts and in sarcoma cell lines (D). The FUS1 protein band was detected using a rabbit anti-FUS1 polyclonal antibody as described in the Materials and Methods. Normal human lung fibroblast WI-38 was assayed as a positive control. The blots were reprobed with anti-β-actin that was used as a loading control. Data are representative of three independent experiments.
sarcoma samples examined displayed detectable expression of the FUS1 protein (Table III and Figure 2B-J) with the exception of three sarcoma cases (4%) that showed patchy positive immunoreactivity with the anti-FUS1 antibody. Of these three cases, one MFH case and one leiomyosarcoma case showed a moderate reaction, while one liposarcoma case displayed a mild reaction (Figure 2B, C and D). None of these three cases showed loss of FUS1 mRNA expression. FUS1 protein expression was also undetectable in benign BSTs (Table III and Figure 2K and L) and in healthy bone and soft tissues except for peripheral nerve tissues (Figure 2M-P). The FUS1 protein was expressed in the axons of peripheral nerve tissues but it was not expressed in any of the myelin sheaths, which are the primary sites of origin of peripheral nerve sheath tumors (Figure 2M). Thus, it appears that FUS1 protein expression is generally absent from both sarcomas and benign BST samples.

**Forced FUS1 gene expression inhibits the growth of sarcoma cells.** To test the hypothesis that FUS1 protein can function as a tumor suppressor in bone and soft tissue sarcomas as it does in other tumor types, the wt-FUS1 gene or the negative control LacZ gene were transiently transfected into eight sarcoma cell lines which exhibited extremely low endogenous FUS1 protein expression or a complete loss of it. FUS1 protein expression was analyzed by Western blotting of cell extracts 48, 72 and...
96 h after exogenous wt-FUS1 gene delivery. This analysis revealed that six out of the eight sarcoma cell lines (NOS10, SaOS2, ASPS-KY, HS-SY-II, NMS-2 and OUMS-27) exhibited strong, time-dependent FUS1 protein expression when transfected with wt-FUS1, but not when transfected with LacZ. The other two FUS1-transfected sarcoma cell lines displayed very low (NMFH2) or undetectable (SKNMC) FUS1 protein expression (Figure 3). In contrast, there was no significant difference in FUS1 protein expression levels between the wt-FUS1-transfected and the control vector LacZ-transfected normal lung fibroblast WI-38 cells.

To examine whether the exogenously expressed FUS1 protein can suppress sarcoma tumor growth, the viability of these eight sarcoma cell lines was analyzed at 24, 48, 72 and 96 h after wt-FUS1 or LacZ-gene transfection, using the XTT assay and assigning a viability value of 1 to each LacZ-transfected control. FUS1 transfection significantly, and in a time-dependent manner, decreased the viability of six of the sarcoma cell lines (NOS10, SaOS2, ASPS-KY, HS-SY-II, NMS-2 and OUMS-27), resulting in viability values relative to LacZ-transfected normal lung fibroblast WI-38 cells of 0.87-0.77, 0.65-0.59, 0.73-0.56, 0.87-0.71, 0.87-0.71, 0.81-0.68 and 0.86-0.74, respectively, 48-96 h after transfection (p<0.01, paired t-test) (Figure 4A). For three of these six sarcoma cell lines (SaOS2, ASPS-KY and NMS-2), significant inhibition of tumor cell growth was observed even as early as 24 h after wt-FUS1 gene transfection, with viabilities of 0.85, 0.83 and 0.89, respectively, compared to the control LacZ group (p<0.01, paired t-test). The obvious cell death mediated by exogenous wt-FUS1 gene transfection into these six sarcoma cell lines was also demonstrated by phase-contrast analysis of cell morphology 72 h post transfection (Figure 4B). However, cell viability analysis did not reveal any statistically significant cell death in the other two sarcoma cell lines (NMFH and SKNMC) after exogenous wt-FUS1 gene transfection compared to LacZ transfection.

Activation of apoptosis by exogenous FUS1 expression. Activation (cleavage) of caspase-3 plays a critical role in the execution of apoptosis (22), and the proteolytic cleavage of PARP by activated caspase-3 serves as a marker of apoptosis (23). The present study, therefore, determined whether the significant suppression of tumor cell growth induced by wt-FUS1 gene transfection is mediated by the induction of apoptosis. Using Western blot analysis, the protein expression levels of cleaved caspase-3 and cleaved PARP were examined in the six sarcoma cells (NOS10, SaOS2, ASPS-KY, HS-SY-II, NMS-2 and OUMS-27), which exhibited strong FUS1 protein expression after transient gene transfection. The cleaved caspase-3 protein product (19/17 kDa) was clearly detected 48 and 72 h after wt-FUS1 gene transfection in five out of these six sarcoma cells (NOS10, SaOS2, ASPS-KY, HS-SY-II and NMS-2) (Figure 5A). However, activation of caspase-3 in the chondrosarcoma OUMS-27 cells was ambiguous at 48 h and was undetectable at 72 h post transfection (Figure 5A). In addition, a strong increase in the expression levels of the cleaved PARP protein (89 kDa) was detected in all six FUS1-transfected sarcoma cells compared with the control LacZ-transfected cells (Figure 5B). Cleavage of the PARP protein observed in the OUMS-27 cells may be mediated by a caspase other than caspase-3, such as by caspase-7 (24).
Figure 4. Exogenous wt-FUS1 gene transfection decreases cell viability and alters cell morphology of bone and soft tissue sarcoma cells. A: Eight sarcoma cell lines were plated on 96-well plates (2×10³/well) and were transfected with a plasmid encoding the wt-FUS1 gene or a control LacZ gene using the FuGENE 6 transfection reagent after 24 h incubation. Cell viability was assessed at 24, 48, 72 and 96 h after transfection using an XTT assay. The viability of the LacZ-transfected cells was regarded as 1.0, and the viability of FUS1-transfected cells was compared relative to this level. Asterisks indicate significant differences at p<0.01. Date are presented as the mean ± standard deviation (n=4). B: Photomicrographs of human sarcoma cells after exogenous wt-FUS1 gene transfection. Cell morphology was observed using a phase-contrast microscope and cells were photographed at a magnification of ×40, 72 h after gene transfection.
**Discussion**

*FUS1*, which is a novel candidate tumor suppressor gene, plays an important role in the earliest steps of lung cancer pathogenesis and progression (9). *FUS1*-deficient mice showed an increased predisposition to a certain range of tumors (25). It is unknown whether *FUS1* also functions as a ‘gate-keeper’ in the pathogenesis of human bone and soft tissue sarcomas. This study provided evidence that *FUS1* may also act as tumor suppressor in human bone and soft tissue sarcomas.

Somatic mutation of the *FUSI* gene is rare in both lung cancer cell lines and in primary lung cancers (<4%) and the gene transcription levels in these lung cancer cell lines and tumor samples are similar to those in normal lung and fibroblast cells (5). The results of the present study are consistent with these previous data. *FUS1* mRNA was not only expressed in all three human normal fibroblast cell lines...
examined, but also in all healthy tissue, all benign BSTs, all sarcoma cell lines and almost all sarcoma tumors analyzed (Figure 1A-C and Table II). These data indicated that loss of FUS1 gene expression is also a rare event in bone and soft tissue sarcomas.

In contrast to FUS1 mRNA expression, it has been shown that endogenous FUS1 protein expression cannot be detected in lung cancer cell lines by Western blotting using FUS1-specific polyclonal antibodies, in spite of its expression in normal human bronchial cells and lung fibroblast cells (8). Loss or reduction of FUS1 protein expression was detected in 82% of non-small cell lung cancer specimens and in 100% of small cell lung cancer specimens using immunohistochemical tissue microarray analysis. However, FUS1 protein expression was not lost in any of the non-malignant bronchial epithelial specimens (9). In the present study, compared with FUS1 protein expression in the normal lung fibroblast WI-38 cell line (positive control), the expression of FUS1 protein was also either very low or completely absent from the sarcoma cell lines tested, although FUS1 mRNA expression was detected in all these cell lines (Figure 1D). In addition, the immunohistochemical analysis detected FUS1 protein expression in only 3 out of 70 sarcoma samples (Table III and Figure 2). However, the results of this study regarding FUS1 protein expression in normal cells, healthy tissues and benign tumor tissues differ from previous studies. This is because FUS1 protein expression was relatively low in two normal fibroblasts that originated from mesenchymal tissues (Figure 1D) and was also absent from all of the benign BST samples and of the various healthy human bone and soft tissues assayed (Table III and Figure 2). Thus, whereas previous observations showed a high level of FUS1 protein expression in healthy lung bronchial epithelia, but a reduced or lost expression in preneoplastic respiratory epithelia and malignant lung tumors (9), in the present immunohistochemical study, the expression of the FUS1 protein was undetectable in both healthy and benign mesenchymal tumor tissues. There are two possible explanations for these results. Firstly, FUS1 may function differently as a tumor suppressor in different tissues. Secondly, FUS1 may not be a major contributor to the tumorigenesis of sarcomas.

The exact mechanism of the inactivation of FUS1 protein expression in primary human carcinomas, including lung and breast cancer, remains unknown. The haploinsufficiency that occurs at the 3p21.3 region where the FUS1 gene resides may result in a decrease in, or loss of, FUS1 protein expression (6, 8, 26). A reduction or loss of FUS1 protein may also result from its rapid proteasome-dependent degradation due to a deficiency in post-translational myristoylation of FUS1 proteins. Alternatively, FUS1 protein translation may be down-regulated after transcription by micro-RNAs that target the 3’UTR of FUS1 (27, 28). Although the exact mechanism by which FUS1 protein expression is inhibited in soft tissue sarcomas is unknown, similar mechanisms of FUS1 down-regulation may also operate in sarcoma cells.

The present study involving transfection of exogenous FUS1 into sarcoma cell lines and showing weak, or no, endogenous FUS1 protein expression, suggested that FUS1 does indeed play a tumor-suppressor function in sarcoma cells. It was shown that six of the eight FUS1-transfected sarcoma cells showed high protein expression of exogenous FUS1 that was accompanied by decreased cell viability compared to LacZ-transfected cells and by induction of apoptosis (Figures 3-5). It is possible that regulation of the protein expression and the activity of FUS1 in sarcomas may differ from those in lung carcinomas. FUS1 protein expression in healthy tissue or in benign BSTs may be silenced at a post-translational stage, and may be activated or up-regulated only in response to oncogenic stresses or apoptotic stimuli. However, the number of healthy tissue samples used in this study was insufficient for an analysis of the reason why FUS1 protein expression is lacking from these tissues.

In conclusion, this study showed the frequent lack of FUS1 protein expression in bone and soft tissue sarcomas and in benign BSTs, as well as in healthy mesenchymal tissues, despite their high FUS1 mRNA expression. FUS1 is a potential tumor suppressor of sarcoma cells and may play an important role in the pathogenesis of sarcomas. Further studies of FUS1 gene and protein expression in a larger number of BSTs and healthy bone and soft tissues, and studies of its biological activity in vitro and in vivo are required to confirm its precise role in these tumors.

References


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