Mitochondrial Proliferation Induces Apoptosis of Human Malignant Fibrous Histiocytoma

INTRODUCTION
Musculoskeletal malignancies, particularly high-grade sarcomas such as malignant fibrous histiocytoma (MFH), are clinically aggressive and have a high metastatic behavior in various organs. Today, there is no generally applied effective treatment other than intensive surgery, therefore, new therapeutic strategies against high-grade sarcomas need to be established.

It has been believed that mitochondria independently divide of other intracellular organelles because they contain their own DNA since the endosymbiosis theory. Previous studies suggest that the mitochondrial proliferation may be an integral part of a cascade of apoptotic events (1). Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) is a multi-functional transcriptional coactivator that regulates the activity of multiple transcriptional factors involved in mitochondrial biogenesis. In particular, PGC-1α regulates transcription of the gene coding for mitochondrial transcription factor A (TFAM), an important gene for mitochondrial biogenesis. Previous studies have linked a decrease in mitochondrial energy metabolism or mitochondrial number to cancer development, and it has been reported that PGC-1α expression is decreased in certain cancers, suggesting that PGC-1α:TFAM/mitochondria pathway may be involved in the pathogenesis of various human malignancies.

The purposes of this study were to examine the relationship between musculoskeletal malignancies and mitochondrial biogenesis, and to evaluate the effect of PGC-1α overexpression on mitochondrial proliferation in MFH cells.

METHODS
Human musculoskeletal tumor tissue samples and human MFH cell line. Thirty-nine tumor samples, including 14 osteosarcomas, 6 MFHs, 7 liposarcomas and 12 schwannomas, were obtained by surgery at Kobe University Hospital in accordance with institutional guidelines. A human MFH cell line, Nara-H, was grown in culture medium and cells were maintained at 37°C in a humidified 5% CO₂ atmosphere.

Quantitative real-time PCR. In 39 tumor samples, we examined mRNA expression of PGC-1α and TFAM by quantitative real-time PCR. We isolated genomic DNA from tumor samples and Nara-H cells, and evaluated the relative amounts of mitochondrial DNA (mtDNA) to nuclear DNA.

Transfection of MFH cells with PGC-1α overexpressing plasmid and/or TFAM siRNA. To evaluate the effects of PGC-1α and TFAM expression in MFH cells, we performed co-transfection of Nara-H cells with PGC-1α plasmid (or control plasmid) and TFAM siRNA (or negative control siRNA) by lipofection method.

Immunoblot analysis. After plasmid/siRNA transfection, we evaluated the expression of PGC-1α and TFAM by immunoblot analysis. To verify if the expression of PGC-1α and TFAM affects mitochondrial apoptosis, we examined the cleavage of caspase-3, -8 and -9 by immunoblot analysis, and the expression of cytochrome c and Bax protein were evaluated in mitochondrial and cytoplasmic fractions.

Evaluation of mitochondrial apoptosis. To investigate the extent of apoptosis following PGC-1α-induced mitochondrial proliferation, we assessed DNA fragmentation and the number of mitochondria in transfected cells by FACS analysis. We also performed immunofluorescence staining to verify the relationship between mitochondrial proliferation and cell apoptosis.

Statistical analysis. Each experiment was performed at least three times independently. Statistical significance was evaluated using student’s t-test and all tests were considered significant at p<0.05.

RESULTS
The amounts of mtDNA and the expression of both PGC-1α and TFAM were decreased in malignant musculoskeletal tumors. Quantitative real-time PCR revealed that, in malignant tumors, the amounts of mtDNA and the expression of both PGC-1α and TFAM were significantly decreased compared with benign tumors (Fig.1.).

PGC-1α overexpression induced mitochondrial apoptosis in human MFH cells via mitochondrial proliferation.

Immunoblot analysis showed that, in PGC-1α plasmid/control siRNA transfected cells, cleavage of both caspase-3 and -9 increased compared with other transfected cells (Fig.2). The cleavage of all three caspases was unchanged in cells that were transfected with TFAM siRNA. We also found that in PGC-1α plasmid/control siRNA transfected cells, the expression of cytochrome c decreased in the mitochondrial fraction and increased in the cytoplasmic fraction. Conversely, Bax expression increased in the mitochondrial fraction and decreased in cytoplasmic fraction. As shown in Fig.3., both FACS analysis and immunofluorescence staining revealed that the number of apoptotic cells increased along with an increase in mitochondrial proliferation in PGC-1α plasmid/control siRNA transfected cells, however, the increase in apoptotic cell death and mitochondrial proliferation were markedly suppressed by TFAM siRNA transfection (Fig.3).

DISCUSSION
In this study, we found that PGC-1α overexpression induced human MFH cell apoptosis via an increase in mitochondrial number in vitro. These results strongly suggest that mitochondria play a crucial role in human musculoskeletal carcinogenesis and that PGC-1α may be a novel molecular therapeutic target for MFH. We believe that regulation of mitochondrial proliferation may provide a breakthrough in anti-tumor therapy for human malignancies.

SIGNIFICANCE
Regulation of mitochondrial proliferation by PGC-1α overexpression induces apoptosis of human MFH.

REFERENCES