A *p53* Gene Mutation in Malignant Fibrous Histiocytoma Associated with Bone Infarction

Yasuhiro Yamamoto,1 Yasunari Takakuwa,2 Makoto Kuroda,2 Hiroatsu Nakashima,3 Yuki Washimi,1 Daisuke Ishimura1 and Harumoto Yamada1

1Department of Orthopaedics Surgery, School of Medicine, Fujita Health University, Toyoake, Aichi, Japan
2Department of Pathology, School of Medicine, Fujita Health University, Toyoake, Aichi, Japan
3Department of Orthopaedics Surgery, Aichi Cancer Center, Aichi Hospital, Okazaki, Aichi, Japan

Transformed sarcomas rarely arise from bone infarct lesions, although the majority of bone sarcomas are primary in origin. However, the pathogenesis of the condition is unknown. In this report, we describe a malignant fibrous histiocytoma with a p53 gene mutation. A 59-year-old woman complained of having pain in her left knee for three months. Plain radiographs of the distal metaphysis of her left femur revealed an ill-defined lytic lesion, which was consistent with a malignant tumor in the infarct lesion. An open biopsy specimen did not show any evidence of malignancy. Immunohistochemical examination of the biopsy specimen failed to show p53 protein-positive cells. However, a mutation in the *p53* gene was detected when polymerase chain reaction/single-strand conformation polymorphism (PCR-SSCP) analysis was performed. A functionally relevant *p53* missense mutation in codon 273 of exon 8 [CGT (Arg) → CAT (His)] was confirmed by direct sequencing. We concluded that this lesion was a malignant bone tumor arising from the bone infarct lesion, and we thus performed a wide resection. The histopathological diagnosis of the resected specimen was that it was a malignant fibrous histiocytoma associated with bone infarction. Immunohistochemistry revealed that the tumor cells were positive for the p53 protein. To our knowledge, our patient is the first patient having a bone infarct-associated sarcoma with a *p53* gene mutation. Identification of the *p53* mutation helps in diagnosing the malignant transformation of the bone infarct lesion. One pathogenesis of this condition may be a mutation in the *p53* gene.

**Keywords:** bone infarct; malignant transformation; p53; polymerase chain reaction/single-strand conformation polymorphism; sarcoma


An infarct-associated sarcoma is uncommon; approximately 70 cases in the world have been filed in the medical literature (Torres and Kyriakos 1992; Domson et al. 2009). The pathogenesis of the malignant transformation remains obscure; however, the reparative tissue adjacent to infarct lesions is the assumed source of the sarcomatous transformation (Mirra et al. 1974, 1977). Bahk and colleagues (2010) report that in their two patients a transition zone consisting of granulation tissues (which are thought to be reparative tissues), including interlacing spindle cell fascicles and capillary proliferation with chronic inflammatory cells – exists in the space between the ancient infarct and the transformed sarcoma. On the sarcoma-side of the transition zone, the atypical spindle cells imperceptibly merge into a frank, high-grade sarcoma. Their data provide actual histological evidence to support the above assumption.

Alterations in tumor suppressor gene *p53* are believed to have a significant role in the initiation, the dedifferentiation process, and the progression of various malignant tumors (Knudson 1993). It is well known that secondary malignant degeneration arises under pre-existing conditions. For example, malignant fibrous histiocytoma arises from the draining sinus of chronic osteomyelitis (Saglik et al. 2001; Foti et al. 2002); skin cancer arises from large burn scars (Rhim et al. 1995; Alconchel et al. 1997; Hayashi et al. 2003); and colic cancer is derived from ulcerative colitis (Fujii et al. 2008). Some conditions are associated with a *p53* gene mutation (Rhim et al. 1995; Fujii et al. 2008). Infarct-associated sarcoma also arises from a pre-existing condition. In our patient, PCR-SSCP detected a *p53* gene mutation in the fibroblast-like spindle cells in the reparative granulation tissue of the transition zone adjacent to the infarct lesion.

**Clinical report**

A 59-year old woman complained of having progres-
sive severe pain in her left knee for three months. Her past medical history was significant for a myocardial infarction and coronary bypass surgery at the age of 40 years. At that time, she was diagnosed as having dyslipidemia. Plain radiographs of both distal femurs revealed symmetrical, ill-defined areas of increased density with patchy sclerosis, which is typical of bone infarcts. Computed tomography (CT) scans of the left femur showed a large ill-defined lytic lesion in the infarct, with cortical destruction (Fig. 1). Magnetic resonance imaging of the left distal femur revealed bizarre signal intensity changes, but an extrasosseous mass was not seen. A bone scan showed increased foci of activity in both distal femurs, especially in the left; however, there was no evidence of other skeletal foci having an increased uptake. CT scans of the chest did not show pulmonary metastasis. An intra-lesional biopsy was performed on the left femur, but the histological specimen revealed the fascicles of fibroblast-like spindle cells with chronic inflammatory cells (Fig. 2A). There was no distinct evidence of a malignancy. We were unable to determine whether this lesion was a malignant bone tumor or represented a chronic reparative process in a bone infarct. PCR-SSCP analysis focused on exons 5 to 8 of the \( p53 \) gene for this biopsy specimen. The analysis detected a mutation on exon 8. A functionally relevant \( p53 \) missense mutation in codon 273 of exon 8 (G-to-A transition) was observed by direct sequencing [CGT (Arg) \( \rightarrow \) CAT (His)], although the immunohistochemical staining for the \( p53 \) protein was negative (Fig. 2B, C). A hereditary mutation could be excluded after investigation of the patient’s blood. Therefore, we diagnosed this lesion as a bone infarct-associated neoplasia.

The patient underwent wide resection of the distal left femur, and reconstruction with a metallic endoprosthesis.

Histologic sections of the lesion that were resected from the left distal femur revealed nonviable bone trabeculae (Fig. 3A [T]) and a fibrous scar lesion (Fig. 3A [S]), which are characteristic of bone infarction. Interlacing non-atypical fibroblast-like spindle cell fascicles with chronic inflammatory cells were adjacent to this infarct area. This was similar to the finding in the biopsy specimen (Fig. 3A, B). As shown in the biopsy specimen, the expression of the \( p53 \) protein was negative in this area (Fig. 3b). The atypical cells dotting the region distal to this area gradually increased in number and (in the more distant region) increased in cellular atypism grades. The atypical cells extended to the spindle-cell sarcomatous area in a storiform pattern (Fig. 4C). In the distal portion, the atypical spindle cells moreover merged into the higher-grade pleomorphic sarcomatous area, which was composed of abundant extremely pleomorphic cells that ranged from the plump spindle cells to the large polygonal cells, bizarre tumor giant cells and atypical mitoses (Fig. 4D). In immunohistochemical studies, expression of the \( p53 \) protein in the pleomorphic sarcomatous area was stronger and more prevalent than its expression in the spindle-cell sarcomatous area (Fig. 4c, d). Maps of the histological distribution of each of the resected specimens are shown in Fig. 5. We finally diagnosed this lesion as a malignant fibrous histiocytoma that was associated with a \( p53 \) gene mutation and arose from the bone infarct lesion. The patient was followed for two years and showed no evidence of recurrence or distant metastasis.
Usefulness of the Investigation of p53 Mutation

Fig. 2. Photomicrographs of the biopsy specimen.

The black arrows indicate the fascicles of fibroblast-like spindle cells and the white arrows indicate chronic inflammatory cells. (A) The spindle cells show no obvious cellular atypia. (B) Immunohistochemical staining for the p53 protein was negative (black bar, 50 μm). (C) However, polymerase chain reaction/single-strand conformation polymorphism (PCR-SSCP) analysis, which focused on exon 5 to 8 of the p53 gene, detected a mutation on exon 8 (a, biopsy tissue; b, normal tissue).

Fig. 3. Histologic sections adjacent to the infarct lesion.

(A) An area of interlacing fibroblast-like spindle cell fascicles (circle) is adjacent to the infarct lesion that consists of (T) nonviable bone trabeculae and (S) fibrous scar lesion. (B) At higher magnification, the proliferation of the spindle cells with chronic inflammatory cells, as well as the biopsy specimen, show no obvious cellular atypism. (b) Immunohistochemical staining for the p53 protein was negative (white bar, 200 μm; black bar, 50 μm).
Fig. 4. Histologic sections of the malignant lesions.

(C) In the distal and postero-lateral portion adjacent to the area containing the interlacing fibroblast-like spindle cell fascicles, a neoplasm is present that is composed of spindle cells arranged in a storiform pattern (arrow). (c) Immunohistochemical staining for the p53 protein is positive. (D) In the adjacent distal-medial component, a high-grade pleomorphic sarcomatous area with atypical mitoses is present (arrow). (d) Immunohistochemical staining for the p53 protein shows that the expression of the protein in this area is stronger and more prevalent its expression in the former area (black bar, 50 μm).

Fig. 5. Sagittal and axial photographs of the gross sections of the left femur show each histologic distribution. B shows the interlacing fibroblast-like spindle cell fascicles area; C, the spindle-cell sarcomatous (i.e., storiform pattern) area; and D, the pleomorphic sarcomatous area. A represents the axial section line; S, the sagittal section line; and ×, the biopsy portion.
**Discussion**

Most primary malignant bone tumors develop *de novo* in normal bone, although they may arise from pre-existing benign bone tumors or under non-tumorous conditions. This is observed in cases of multiple hereditary exostosis, Ollier’s disease, synovial chondromatosis, fibrous dysplasia and Paget’s disease, and after radiation therapy. Bone infarcts are included among these benign lesions. Sarcomas arising from bone infarcts are extremely rare; however, they are well-described entities. Common types of bone infarction-associated sarcoma are malignant fibrous histiocytoma (Torres and Kyriakos 1992), osteosarcoma (Resnik et al. 1993), fibrosarcoma (Furey et al. 1960), and angiosarcoma (Abdelwahab et al. 1992). The pathogenesis of this disease is not established, although the reparative tissue adjacent to an infarct is an assumed source of the sarcomatous transformation (Mirra et al. 1974, 1977; Kitano et al. 1984). Bahk and colleagues (2010) recently performed a histological investigation of two cases of infarct-associated sarcoma and reported that the transition zone consisted of granulation tissues (including interlacing spindle cell fascicles). Capillary proliferation with chronic inflammatory cells was adjacent to the ancient infarct. Atypical spindle cells imperceptibly merged into the high-grade spindle cell sarcoma on the sarcoma side of the transition zone. Bahk and colleagues (2010) assume that this transition zone is reparative tissues at the periphery of an ancient infarct and may be a source of the sarcomatous transformation.

On the other hand, alterations in tumor suppressor gene *p53*, which is localized on chromosome 17p13, are believed to play a significant role in the initiation, dedifferentiation process, and progression of various malignant tumors (Nigro et al. 1989; Knudson 1993). It is well known that secondary malignant degeneration may arise under pre-existing conditions. For example, malignant fibrous histiocytoma may arise from the draining sinus of chronic osteomyelitis (Saglik et al. 2001; Foti et al. 2002); skin cancer may arise from large burn scars (Rhim et al. 1995; Alconchel et al. 1997; Hayashi et al. 2003); and colitic cancer may arise from ulcerative colitis (Fujii et al. 2008). The rate of *p53* gene alteration is high in these malignant degenerations arising under their respective pre-existing condition (Rhim et al. 1995; Fujii et al. 2008). Based on the assumption by both Mirra and Bahk (Mirra et al. 1974, 1977; Bahk et al. 2010), reparative tissue in a bone infarct may create the suitable pre-existing condition for these lesions. In our patient, histological examination of the resected specimen confirmed that the biopsy specimen contained granulation tissues that existed in the space between the ancient infarct and the transformed sarcoma. The granulation tissues included fibroblast-like spindle cell fascicles with chronic inflammatory cells. We believed this tissue corresponded to the “transition zone,” described by Bahk, that may be the origin of a sarcomatous transformation in a bone infarct. Detection of a *p53* gene mutation from the granulation tissues supports Bahk’s assumption that this tissue may be the source of the sarcomatous transformation on molecular biologically. No one has reported that the *p53* gene mutation is correlated with bone infarct-associated sarcomas. However, the mutation may play a critical part in the malignant transformation under this condition.

Moreover, immunohistochemical staining of the *p53* protein and screening for the *p53* mutation using PCR-SSCP are useful in the adjuvant diagnosis of the aforementioned lesions (Fuji et al. 2008), if it is difficult to discriminate neoplasia from regenerative tissues in a histological diagnosis. In bone infarct-associated sarcomas, as well as in the aforementioned lesions, it may be difficult to make an accurate pathological diagnosis (i.e., determining whether it is a neoplasia or regenerative tissue) by using a biopsy specimen since some areas in the same lesion show a variety of histological findings, ranging from reparative granulation tissue to high-grade sarcoma. In a histological diagnosis of a biopsy specimen, detecting a *p53* gene mutation may therefore be a useful method to make an accurate diagnosis if it is not possible to discriminate a neoplasia from reparative tissue.

Several authors, however, have reported that some pre-malignant (and even benign) tumors contain the *p53* mutation (Ögmundsdóttir et al. 2009; Joanna et al. 2010). The mutation therefore does not always prove the existence of a malignancy. The diagnosis of a bone infarct-associated sarcoma is really a clinico-radiographic-pathologic diagnosis (Abdelwahab et al. 1992). As seen in this patient, pain is the most common symptom. Radiologic evidence of malignancy with an underlying bone infarct is an important finding in this condition (Mirra et al. 1977; Torres and Kyriakos 1992; Desai et al. 1996). Therefore, it is important that the final diagnosis of malignancy should be determined using other clinical data.

In conclusion, our data showed that infarct-associated sarcomas may be derived from the chronic reparative tissue of a bone infarct on molecular biologically. The *p53* gene mutation may be one pathogenesis of this condition, and detecting the mutated gene could be a useful method for an accurate diagnosis.

**Conflict of Interest**

The authors have no conflict of interest associated with this article.

**References**


