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CASE REPORT

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Mutation of p53 with loss of heterozygosity in the osteosarcomatous component of a dedifferentiated chondrosarcoma

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Abstract We investigated a dedifferentiated chondrosarcoma of a 61-year-old woman with an osteosarcomatous high-grade component for p53 alteration. The low-grade cartilaginous and the high-grade osteosarcomatous components of the tumor were macrodissected and evaluated separately by immunohistochemistry and molecular biology. We used PCR-SSCP analysis and direct sequencing to screen exons 4–8 for p53 mutations. The p53 intron 1-polymorphism was investigated for loss of heterozygosity. A functionally relevant p53 missense mutation in codon 193 of exon 6 (A-to-T transversion) with loss of wild-type allele was detected only in the dedifferentiated component. Using the monoclonal antibody DO-1, immunohistochemistry failed to show p53 overexpression. This evidence of p53 mutation may be regarded as at least a co-factor that "switched" the preexisting low-grade conventional chondrosarcoma to a highly malignant dedifferentiated tumor.

Key words Dedifferentiated chondrosarcoma · p53 mutation · p53 LOH

Introduction

So-called dedifferentiated chondrosarcoma is a rare variant, accounting for about 10% of all chondrosarcomas [6]. After some sporadic cases had been described by O'Neal and Ackerman [11] and Jaffe [8], Dahlin and Beabout [4] introduced the term 'dedifferentiated chondrosarcoma' to describe a primary bone tumor composed of low-grade cartilaginous areas associated with, but sharply delineated from, high-grade sarcomatous areas. The latter most frequently show histological features typical of fibrosarcoma, malignant fibrous histiocytoma, or osteosarcoma. The origin of nonchondroid component is still unclear. While some authors suppose that genomic instability of chondroid tumor cells causes 'dedifferentiation' of the tumor in a sense of tumor progression [6], other investigators think that two separate cell clones develop from multipotent mesenchymal stem cells that are the origins of both parts [1, 20]. Clinically, the noncartilaginous high-grade component determines growth, the formation of metastases, and the poor prognosis of the neoplasm.

There is increasing evidence that tumor suppressor gene mutations play a major part in the development and/or progression of malignant tumors, with p53 mutations occurring frequently in the oncogenesis of various mesenchymal neoplasms [9]. It is well reported that chondrosarcomas accumulate p53 mutations and LOH in the course of tumor progression (Table 1).

In the tumor investigated by us, we were able to separate the highly malignant tumor areas from the low-malignancy component by macrodissection. We investigated the potential role of p53 alterations in the dedifferentiation process of chondroblastic tumors.

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Case report

The patient, a 61-year-old woman, was admitted to the Orthopedic Department, Magdeburg University Hospital, with a pathological fracture of the proximal femur. Radiology revealed a highly aggressive osteolytic tumor with a soft tissue mass in the proximal diaphysis of the left femur, for which the patient had to undergo surgery. Frozen sections of the tumor showed structures of a low malignant conventional chondrosarcoma (CSA) in association with an anaplastic sarcoma, with production of tumor osteoid in parts (Figs. 1, 2). Therefore, the diagnosis of an osteosarcomatous dedifferentiated CSA was made, which was later confirmed by histological examination of paraffin-embedded material. The proximal femur was resected and an endoprosthesis was implanted, followed by adjuvant chemotherapy.

Table 1 Summary of the p53 point mutations in chondrosarcoma reported in the literature (*n.d.* not done, *n.s.* not specified, *CS* chondrosarcoma, *I-III* grade I–III conventional chondrosarcoma, *V* chondrosarcoma variants including clear cell, mesenchymal, and extraskeletal myxoid, *CC* clear cell chondrosarcoma, *DD* dedifferentiated chondrosarcoma)

Reference	Type	p53 mutation	Exon	Codon	LOH
[5]	7 I, 2 II, 1 III, 6 V	2/14 (1 III, 1 III CC)	5, 8	157, 273	n.d.
[23]	1 DD	1/1 DD	8	294	n.d.
[12]	18 n.s. CS	0/18	–	–	n.d.
[24][a]	13 I, 10 II, 1 III, 4 DD	5/28 (1 III, 4 DD)	4, 5, 5, 7, 9	135, 162, 173, 249, 316-7	4/5
[19]	12 I-III, 13 V, 14DD, 7 M, 2 B	1/31 (1 DD)	8	276	n.d.
Current report	1 DD	1/1 DD	6	193	1/1

[a] Results partly published in [21]

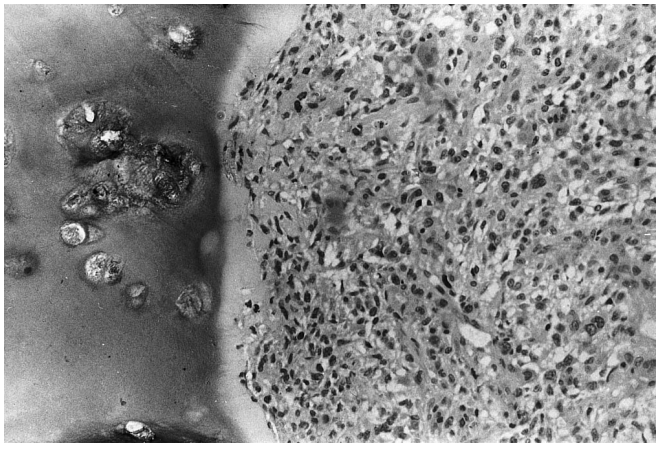

Fig. 1 Juxtaposition of low malignant conventional CSA and anaplastic tumor components. H&E, 200×

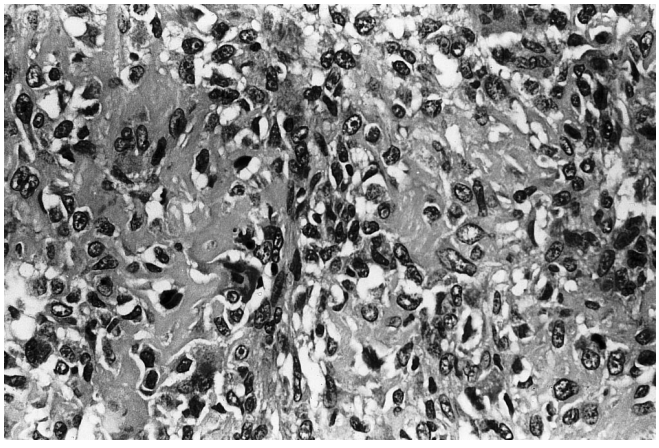

Fig. 2 High magnification of the anaplastic component of the tumor with production of tumor osteoid. H&E, 400×

Materials and methods

Tissue specimens (T) were taken from three macroscopically different areas of the tumor and designated T1, T2, and T3. For immunohistochemistry, the tumor tissue was fixed in formalin and embedded undecalcified in paraffin. Sections were stained with hematoxylin-eosin (H&E) and elastica-van Gieson (EvG). P53-expression was detected by the monoclonal antibody DO-1 (Oncogene Science, Hamburg, Germany) and the alkaline phosphatase/anti-(alkaline phosphatase) technique as previously described by Schneider-Stock et al. [15]. The monoclonal antibody DO-1 reacts with the wild-type p53 and the mutant forms of the protein (residues 21–25 of human p53). The tumor was also stained with the polyclonal antibody anti-S100 protein (Dako, Hamburg, Germany; dilution 1:10,000) and the monoclonal antibody anti-vimentin (Dako; dilution 1:20) by using the ABC (avidin–biotin complex) method with alkaline phosphatase and new fuchsin as chromogenic substrates (Vectastain, Vector Laboratories, Burlingame, Calif.). Detection was done according to the manufacturer's recommendations.

For the molecular genetic analysis, the three tumor parts designated T1, T2, and T3 were snap-frozen in liquid nitrogen and stored at –80°C.

For DNA preparation, tumor DNA from T1, T2, T3 and the blood of the patient were prepared separately by using the proteinase K–phenol–chloroform extraction method according to a protocol of Sambrook et al. [14].

For PCR-SSCP sequence analysis conservative regions of the p53 gene (exons 4–8) were amplified and screened by the PCR and SSCP technique as recently described by Schneider-Stock et al. [15, 16]. PCR fragments showing aberrant single strands were sequenced on an automated fluorescence sequencer [ALF Express (Pharmacia Biotech, Freiburg, Germany) or ABI 373 (Perkin Elmer Cetus, N.J.)] to confirm mutations.

For p53-LOH analysis, allelic losses at the p53 gene were determined by using two intragenic polymorphic markers (intron 1, exon 4) according to a protocol devised by Schneider-Stock et al. [17]. A loss of heterozygosity (LOH) was considered positive if the signal of a tumor band disappeared or was significantly reduced in comparison to the band in the corresponding blood DNA.

For allele-specific oligonucleotide hybridization (ASO), exon 6 fragments of the tumors and the blood genomic DNA were heat-denatured and immediately stored on ice. All four samples (2 µl each) were spotted onto a nylon membrane, dried and UV-cross-linked for 5 min. Probe labeling and detection were done according to the manufacturer's instructions (Gene Images, CPD Star, Amersham, Braunschweig, Germany).

The wild-type probe was K193WT 5'-GGCCCCTCCTCAG CATCTTATCCGAGT-3' and the mutant probe, K193Mut 5'-GGCCCCTCCTCAGCTTCTTATCCGAGT-3'.

Results

Histology and immunohistochemistry

Histological examination of the tumor specimens designated T1, T2, and T3 revealed that T1 consisted of the cartilaginous component of the dedifferentiated chondrosarcoma contaminated by very small portions of the juxtaposed anaplastic tumor. T2 showed a balanced quantity of both

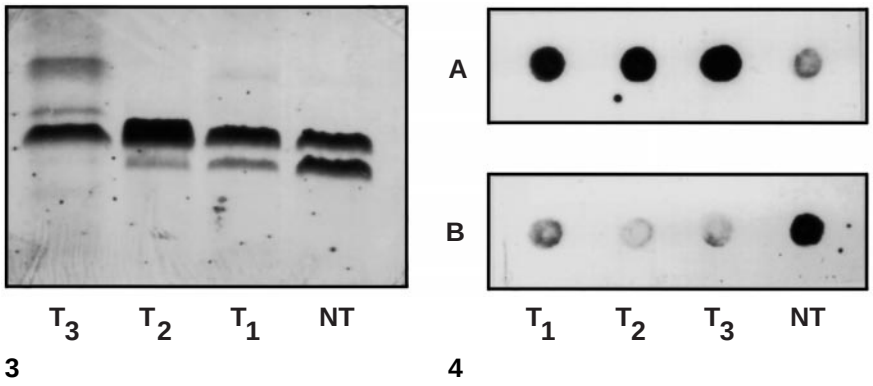

Fig. 3 Loss of heterozygosity (LOH) in the different tumor components of the chondrosarcoma demonstrated by microsatellite analysis of intron 1 of the p53 gene. In contrast to the LOH in T3, the tumor samples T2 and especially T1 showed no clear LOH with almost normal allele bands in the latter. Since histology of all tumor probes revealed no contamination with non-neoplastic tissue, this finding corresponds with the well-differentiated chondroblastic tumor component present in T2 and, to a higher degree, in T1

Fig. 4 Dot-blot with **A** mutant-specific and **B** wild-type-specific oligonucleotides of exon 6 and PCR products of the tumor samples designated T1, T2, and T3 and of blood of the patient. T1 consisted of the cartilaginous component of the dedifferentiated chondrosarcoma contaminated by very small portions of the juxtaposed anaplastic tumor. T2 showed a balanced quantity of both cartilaginous and anaplastic tumor parts, whereas T3 consisted solely of the anaplastic tumor. The signals of mutated p53 (**A**) decrease from T3 to T1 whereas the signals of wild-type p53 (**B**) increase in the same order

cartilaginous and anaplastic tumor parts (Fig. 1), whereas T3 consisted solely of the anaplastic tumor (Fig. 2). Immunohistochemically, S-100 expression was mostly restricted to the cartilaginous component of the tumor. Neither in the cartilaginous nor in the osteosarcomatous component was p53 overexpression recognized immunohistochemically.

Molecular biology

PCR-SSCP sequence analysis of the three separately prepared tumor parts showed that T3 was the only specimen with a definite band shift in the SSCP gel. Sequence analysis revealed a p53 missense mutation in codon 193 of exon 6 (transversion of A to T) with loss of the wild-type allele in T3 (anaplastic tumor component) (Fig. 3). A hereditary mutation could be excluded after investigation of the patient's blood. In contrast to the finding in T3, the tumor samples T2 and, in particular, T1 showed no clear LOH, with almost normal allele bands in the latter. Since histological examination of all tumor probes revealed no contamination with nonneoplastic tissue, this finding corresponds with the well-differentiated chondroblastic tumor component present in T2 and, to a higher degree, in T1.

This hypothesis was verified by allele-specific oligonucleotide hybridization in dot-blot. It showed signals of wild-type p53 increasing from T3 to T1, whereas the signals of mutated p53 decreased in the same order (Fig. 4).

Discussion

We present a case of dedifferentiated chondrosarcoma with point mutation (exon 6, codon 193, CAT to CTT) restricted to the high-grade osteosarcomatous component and not present in the low-grade cartilaginous part. This p53 mutation was found to be functionally relevant, because LOH analysis showed loss of heterozygosity on 17p. The monoclonal antibody Do-1 failed to show overexpression of p53. To date, the following three transversions at codon 193 have been described for mesenchymal tumors: CAT to TAT in liposarcoma [2], CAT to CAA in osteosarcoma [21], and CAT to CTT in uterine sarcoma [10]. In a tumor cohort of 92 chondrosarcomas published so far, 67% of all p53 mutations (9 cases) were found to occur in dedifferentiated tumors (Table 1). In these studies, all the remaining cases of nondedifferentiated chondrosarcomas with mutations of the p53 gene were high-grade tumors. Therefore, the low overall frequency (10%) of p53 mutations in chondrosarcomas most probably reflects the typically low portion of high-grade tumors among conventional chondrosarcomas, with mutations in low-grade tumors being an uncommon event.

To our knowledge, only one dedifferentiated chondrosarcoma has been reported in which the two different tumor portions were separately analyzed at the molecular genetic level (described in [24] for p53-LOH and in [22] for p53 mutations). These authors report a case of a dedifferentiated chondrosarcoma with p53 point mutation (exon 7, codon 249, Arg to Thr) and LOH on 17p only in the high-grade MFH-like component, and not in the low-grade chondroblastic part. Technical difficulties in the separate analysis of different portions of one tumor appear to be the main problem in such molecular biological studies. Microdissection techniques will presumably ease this issue in the future.

In the case presented here, we used macrodissection to separate the different components of an osteosarcomatously dedifferentiated chondrosarcoma. Analysis of p53 mutation in the separately prepared tumor components revealed a p53 missense mutation in codon 193 of exon 6 (transversion of A to T) in the osteosarcomatous but not in the cartilaginous tumor component. We used ASO

with mutant-specific exon 6 PCR products to confirm the mutation in the dedifferentiated component, but failed to show p53 overexpression immunohistochemically. Some previous studies analyzed the different portions of dedifferentiated chondrosarcoma using immunohistochemical techniques. Increased p53 staining was found in the spindle-cell component of 16 of 30 dedifferentiated chondrosarcomas [3, 18, 19, 22]. In only 14 of these was the cartilaginous component of the tumor available for examination. Only the three cases containing a high-grade cartilaginous component were p53 positive [18].

In contrast to many other sarcomas, chondrosarcoma is one of the tumors with a defined histological grading, and the clinical prognosis has been shown to be closely associated with this classification [7]. Our findings support the hypothesis that p53 gene mutations play a part in advanced disease progression, but not in the early tumorigenesis of chondrosarcoma. Thus, the p53 mutation pattern in chondrosarcomas differs from the recently published findings in osteosarcomas, where p53 mutations were found at the same frequency in low- and high-malignancy variants [13].

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