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Short Communication

Simultaneous Interphase Cytogenetic Analysis and Fluorescence Immunophenotyping of Dedifferentiated Chondrosarcoma

Implications for Histopathogenesis

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Cytogenetic analysis of four specimens (biopsy, definitive surgical, and two separately occurring lung metastases) of a dedifferentiated chondrosarcoma with a rhabdomyosarcomatous component revealed clonal karyotypic abnormalities in each. Anomalies seen in all specimens included a structurally aberrant chromosome 17 and extra copies of chromosomes 5, 7, 12, and 20. The derivation of the chromosomally abnormal cells was determined by a combined immunocytochemical/cytogenetic approach that allowed simultaneous assessment of cytogenetic aberrations and immunophenotypic features of individual cells. S-100 protein and desmin antibodies were used to evaluate the chondrosarcomatous and rhabdomyosarcomatous components, respectively. A chromosome 7-specific centromeric probe was used for determination of aneuploidy. In both specimens obtained from the primary lesion, S-100 protein and desmin-positive and-negative aneuploid cells were observed. These findings: 1) suggest that both the chondrocytic and rhabdomyoblastic cells arose from the same abnormal clone, 2) support the theory of a common primitive mesenchymal cell progenitor with the ability to differentiate or express features of more than one line of mesenchymal differentiation, and 3) indicate that the term dedifferentiated may be an inaccurate designation for this neoplasm. (Am J Pathol 1994, 144:215–220)

Cytogenetic data have traditionally been obtained through the microscopic analysis of chromosomes from cells arrested in metaphase. Recent advances in molecular biology have made it possible to detect chromosomal aberrations in the interphase or resting cell using chromosome-specific probes with *in situ* hybridization detection methodologies.[1] A combined molecular cytogenetic/immunocytochemical approach is a valuable form of analysis for further characterizing the neoplastic cell of interest.[2,3] With this technique, the histological lineage of a specific abnormal clone can be determined.

Cytogenetic studies of chondrosarcomas are few.[4] Clonal abnormalities have reportedly been recognized in only two other dedifferentiated chondrosarcomas, neither with a rhabdomyosarcomatous component.[5] The term dedifferentiation refers to the ability of a differentiated cell to redirect to an embryonic, dedifferentiated state.[6] This concept, with respect to dedifferentiated chondrosarcoma, has been controversial. In this study, representative tumor tissue from biopsy, resection, and metastatic speci-

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mens of a dedifferentiated chondrosarcoma were obtained for cytogenetic and/or molecular cytogenetic analysis with simultaneous immunophenotyping to gain a better understanding of the histopathogenesis of this neoplasm.

Clinical History

A radiographically destructive lesion involving the proximal tibia with evidence of punctate calcification and an intra-articular pathological fracture of the lateral plateau was seen in a 56-year-old white female presenting with a 2 year history of progressive asymmetric swelling of the right knee. Microscopic evaluation of an open biopsy specimen revealed cartilage cells of varying size and shape arranged in lobules with focal myxoid change. Two or more cells were frequently seen in individual lacunae. The lesion was interpreted as a grade 2 chondrosarcoma.

A limb-preserving procedure was originally designed. Frozen section analysis of an intraoperatively encountered anterior extraosseous component revealed a high grade spindle-cell sarcoma. The diagnosis was modified to dedifferentiated chondrosarcoma. Subsequently, an above the knee amputation was performed. Grossly, the lobular, gray-white neoplasm involving the proximal tibia with extension into the adjacent soft tissue measured 4.0 × 8.0 × 12.0 cm. The resection margin, distal femur, and joint space were free of tumor. Spindle-shaped cells with deeply eosinophilic cytoplasms comprised much of the neoplasm. Cytoplasmic cross-striations were distinct in some of these cells, and foci of chondrosarcoma similar to the biopsy specimen were jux-

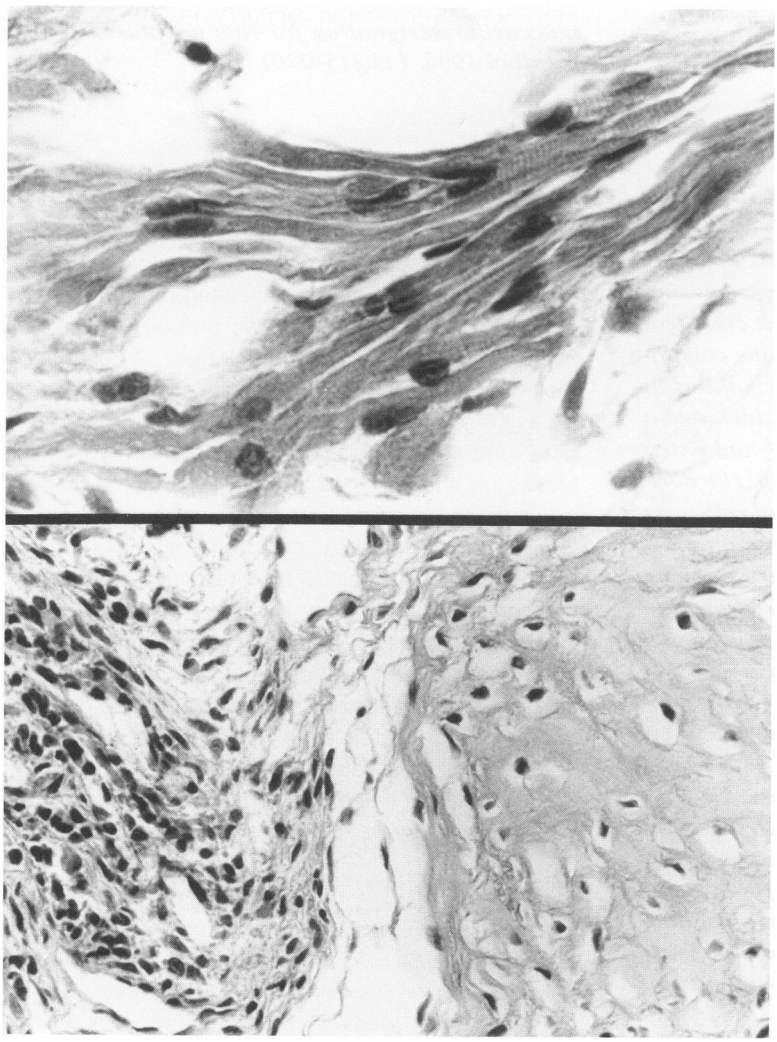

Figure 1. Top: *Distinct cross-striations in many of the tumor cells of the rhabdomyosarcomatous component. (hematoxylin and eosin, ×550).* Bottom: *Transition between the rhabdomyosarcomatous (left) and the chondrosarcomatous (right) components is abrupt. (hematoxylin and eosin, ×340).*

taposed to the rhabdomyosarcomatous elements (Figure 1). Immunohistochemical features supported the diagnosis of dedifferentiated chondrosarcoma with a rhabdomyosarcomatous component (S-100 protein positivity in the cartilaginous regions, and muscle-specific actin and desmin positivity in the rhabdomyosarcomatous). Retrospective examination of the biopsy specimen revealed one small focus of crushed spindle-shaped cells (dedifferentiated chondrosarcoma).

One month after the primary resection, wedge resections were performed on the left upper and lower lung lobes to remove metastatic lesions. Both lesions were well demarcated and firm measuring 0.9 × 0.9 × 0.9 cm and 0.1 × 0.2 × 0.2 cm (upper and lower lobe lesions, respectively). Histopathologically, these specimens consisted solely of the rhabdomyosarcomatous component. Seven months later, another lung metastasis histologically similar to the previous one was removed (lower left lobe, 1.0 × 1.5 × 2.5 cm).

The patient remains alive with metastatic disease 13 months after the original diagnosis.

Material and Methods

Cytogenetic

The cytogenetic techniques used for chromosomal analysis have been described previously. Briefly, aseptically collected representative 1-cm^3 tumor samples obtained from the biopsy, the resection specimen, and metastatic lung lesions were cultured for 4 to 6 days. Twenty metaphase cells banded with Giemsa trypsin were analyzed for each specimen. Karyotypes were expressed in accordance with the International System for Chromosome Nomenclature.[8]

In Situ *Hybridization and Immunophenotyping*

The fluorescence *in situ* hybridization (FISH) methods and findings used to define the additional material on the short arm of chromosome 17 have been previously described.[4]

Fluorescence interphase cytogenetic analysis and immunophenotyping were performed simultaneously on the biopsy and definitive surgical specimens. Antibodies examined included monoclonal mouse anti-human desmin and rabbit polyclonal anti-bovine brain S-100a (DAKO, Inc., Carpinteria, CA). Prepared cytospins from cultured and/or uncultured tumor cells were air-dried, fixed in acetone, and immunophenotyped with either desmin or S-100 protein and TRITC-conjugated rabbit anti-mouse immunoglobulins (DAKO) or CY-3-conjugated affinipure goat anti-rabbit immunoglobulins (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). The immunophenotyped slides were subsequently emersed in Carnoy's fixative,

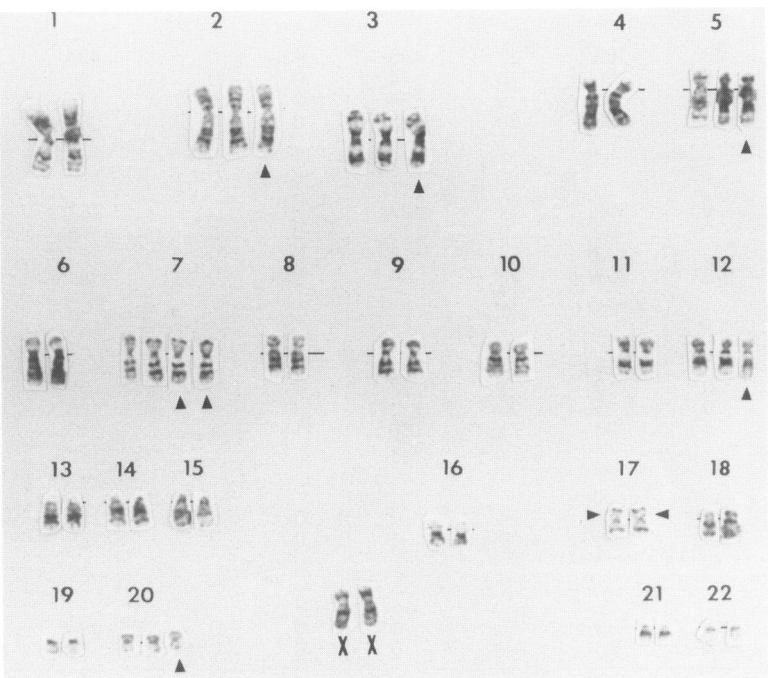

Figure 2. *Representative karyotype of the biopsy specimen: 53,XX,+2,+3,+5,+7,+7,+12, add(17)(p13)×2,+20.*

washed in distilled water (dH_2O), fixed again in 1% paraformaldehyde (2X standard saline citrate[SSC], pH 7.6), and rinsed with dH_2O before dehydration for chromosomal FISH. Also, slides from each specimen were processed as above, however, without the primary antibody. No immunoreactivity was observed. Moreover, a slide of each sample was processed using UNHL-1, a T cell monoclonal antibody (DAKO), for the initial incubation. Nonspecific binding was absent in both specimens.

Chromosomal FISH was executed with a hybridization mixture containing 65% formamide, 10% dextran sulfate, 2X SSC, 0.05 μg/μl sonicated herring testes DNA, and 1 μg/ml biotinylated DNA cocktail probe for the α-satellite regions of chromosome 7 (D7Z1, D7Z2) (ONCOR, Gaithersburg, MD). The hybridized slides were washed three times in three changes of 50% formamide (2X SSC, pH 7.0) at 48 C and detected using fluorescein-avidin DN and biotinylated antiavidin (5 μg/ml) (Vector Laboratories, Burlingame, CA). A Zeiss Axiophot microscope with the appropriate filters (00 for TRITC and CY-3, 09 for FITC, and FITC/Texas red dual band) was used for microscopic evaluation. Approximately, 500 cultured and/or uncultured tumor cells were analyzed for each specimen. An identical number of cells were analyzed for the identically processed control specimens (normal skeletal muscle and nerve).

Results

Cytogenetic

Cytogenetic analysis of the biopsy specimen revealed the following abnormal complement in all of the cells analyzed: 53,XX,+2,+3,+5,+7,+7,+12, add(17)(p13)×2,+20 (Figure 2), and analysis of the definitive surgical specimen showed the following in 15 of 20 cells analyzed: 55,XX,idem,+19,+20. Analysis of the separately occurring lung metastases showed the following abnormal complements, respectively: 52,XX,der(4)t(4;13)(p16;q14),+5,+7, +7,+12,add(17)(p13)×2,+20,+20 and 54,XX,der (4)t(4;13)(p16;q14),+5,+7,+7,+8,+12,add(17) (p13)×2,+19,+20,+20. Chromosomal abnormalities shared by all four specimens included extra copies of chromosomes 5, 7, 12, and 20, and a structural anomaly of 17p.

Table 1. *Immunocytochemical/Cytogenetic Results*

	No. of chromosome 7 signals							Total no. of cells
	0	1	2	3	4	5	6	
Biopsy specimen (uncultured cells)								
S-100 +	0	13	189	33	195	4	0	434
S-100 –	0	0	30	6	44	0	0	80
Desmin +	3	2	69	6	34	0	1	115
Desmin –	0	11	186	39	120	6	0	362
Biopsy specimen (cultured cells)								
S-100 +	3	9	302	14	127	2	0	457
S-100 –	1	2	62	3	10	0	0	78
Desmin +	0	2	143	4	18	0	0	167
Desmin –	2	5	298	22	54	0	0	381
Definitive surgical specimen (uncultured cells)								
S-100 +	4	1	45	73	291	7	0	421
S-100 –	2	0	10	8	32	0	0	52
Desmin +	0	0	25	20	120	3	0	168
Desmin –	0	4	96	71	166	11	1	349
Normal skeletal muscle (uncultured cells)								
Desmin +	3	9	233	4	0	0	0	249
Desmin –	0	9	255	0	1	0	0	265
Normal nerve (uncultured cells)								
S-100 +	4	48	433	7	1	0	0	493
S-100 –	0	4	50	0	0	0	0	54

In Situ *Hybridization*

FISH analysis of 80 metaphase cells with chromosome-specific 1, 3, 13, and 17 painting probes revealed that the additional material on the short arm of chromosome 17 was not derived from any of these chromosomes. Importantly, these findings excluded the possibility of involvement of chromosome 1 (seen as the only shared anomaly between the two previously cytogenetically described dedifferentiated chondrosarcomas[5]) or the presence of an isochromosome 17q (considered as the most likely on the basis of G-banding analysis), as well as involvement of chromosomes 3 and 13.

Concomitant analysis for increased copies of chromosome 7 and desmin or S-100 protein on cultured and/or uncultured tumor cells revealed a significant number of both desmin and S-100 protein positive and negative aneuploid cells (Table 1, Figure 3). The chromosome 7 copy number for the control specimens (normal skeletal muscle and peripheral nerve) is also provided in Table 1.

Discussion

Cartilaginous neoplasms represent nearly one-half of all primary mesenchymal bone tumors. Dedifferentiated chondrosarcoma, also referred to as spindle cell chondrosarcoma, chondrosarcoma with additional mesenchymal component, and chondrosarcoma with a double phenotypic pattern, is a rare

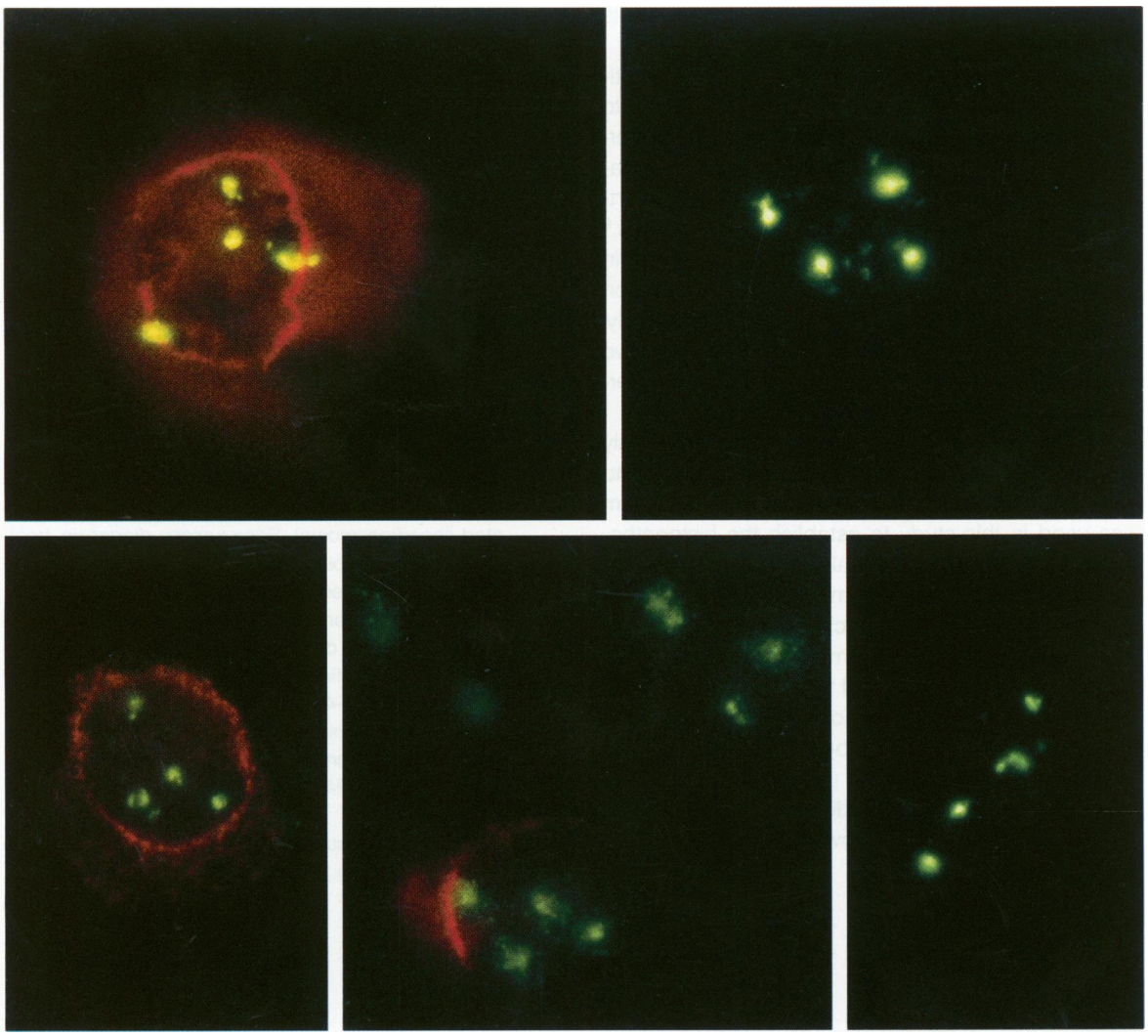

Figure 3. Upper left: *S-100 protein-positive aneuploid cell. The cytoplasmic portion of the cell is immunoreactive for S-100 protein (red) and four signals (green) are seen representative of the α-satellite region of chromosome 7.* Upper right: *S-100 protein-negative aneuploid cell. Only the four signals (green) representative of chromosome 7 copy number are detected in this cell.* Lower left: *Desmin-positive aneuploid cell.* Lower middle: *A desmin-positive and -negative aneuploid cell are both seen in this field.* Lower right: *Desmin-negative aneuploid cell.*

histological subtype. Dahlin and Beabout[9] first proposed the term dedifferentiated chrondrosarcoma when they described 33 patients with well-differentiated chondrosarcoma in which additional malignant mesenchymal elements such as fibrosarcoma or osteosarcoma were also present. The concept of "dedifferentiation" with respect to this form of chondrosarcoma is controversial. Dedifferentiation implies reversal of a differentiated cell to an embryonic, undifferentiated cell. This notion is not consistent with the brief clinical course many patients with such high grade malignant lesions exhibit from the onset, precluding the long-term presence of a preexistent low-grade malignant cartilage tumor. Moreover, the histological identification of a rhabdomyosarcomatous component in addition to a chondrosarcoma (ie, a chondrosarcoma that dedifferentiated into a rhabdomyosarcoma) further discredits this hypothesis.[5]

The number of cartilaginous tumors cytogenetically analyzed is limited, particularly with respect to dedifferentiated chondrosarcoma.[4] Only two dedifferentiated chondrosarcomas, both with fibrosarcomatous and osteosarcomatous elements, have reportedly demonstrated clonal karyotypic aberrations.[5] Multiple complex abnormalities were observed in both tumors; involvement of the same breakpoint on chromosome 1 (1p36) was the only shared anomaly. There were no abnormalities common to these two cases and the present case; however, the dedifferentiated components were also distinct.

A recently described technique[2] simultaneously analyzing the immunohistochemical properties and chromosomal anomalies of a particular neoplasm was used in this study to establish the histopathogenic origin of the clonally abnormal cells. Specifically, S-100 protein and desmin antibodies were examined concurrently with a chromosomal probe for the α-satellite region of chromosome 7 (seen in four copies instead of the normal two in the tumor cells) to determine whether the clonally aberrant cells were of chondrocytic or rhabdomyoblastic origin or both. These analyses were performed before and after standard culture procedures to prevent biased results (selective culture growth of one component or the other).

In both the biopsy and the definitive surgical specimen, S-100 protein and desmin-positive and -negative aneuploid cells were observed. These data suggest that both the chondrocytic and rhabdomyoblastic cells were derived from the same abnormal clone. In other words, this neoplasm arose from a single abnormal clone or cell. We propose a common primitive mesenchymal cell progenitor, possessing the ability to differentiate or express chondrocytic or rhabdomyoblastic features. Thus, these studies provide additional evidence contesting the premise of dedifferentiation in this form of chondrosarcoma.

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