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MOLECULAR GENETIC CHARACTERIZATION OF BOTH COMPONENTS OF A DEDIFFERENTIATED CHONDROSARCOMA, WITH IMPLICATIONS FOR ITS HISTOGENESIS

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SUMMARY

Dedifferentiated chondrosarcoma is defined as a high-grade, anaplastic sarcoma adjacent to a low-grade malignant cartilage-forming tumour. Controversy remains as to whether the anaplastic and cartilaginous components are derived from a common precursor cell, or whether they represent separate genotypic lineages (collision tumour). Both components of a case of dedifferentiated chondrosarcoma were therefore separately investigated by loss of heterozygosity (LOH) analysis, comparative genomic hybridization (CGH), DNA flow cytometry, and p53 analysis. Both showed p53 overexpression and an identical somatic 6 bp deletion in exon 7 of p53. Combination of the CGH and LOH results revealed that both components had lost the same copy of chromosome 13. These results provide compelling evidence in this case for a common origin, instead of the 'collision tumour' theory. Certain genotypic alterations were not shared. The anaplastic component showed severe aneuploidy, LOH at additional loci, and amplification and deletion of several chromosome parts. In contrast, the cartilaginous component had lost chromosomes 5, 22, 17p and part of 16p and revealed an amplification of 17q. The LOH and CGH results further demonstrated that the two components had lost a different copy of chromosome 4. Thus, a substantial number of genetic alterations have occurred after the diversion of the two components, indicating that the separation of the two clones, derived from a single precursor, was a relatively early event in the histogenesis of this case of dedifferentiated chondrosarcoma.

KEY WORDS—**dedifferentiated chondrosarcoma; bone neoplasm; loss of heterozygosity; comparative genomic hybridization; p53**

INTRODUCTION

The term dedifferentiated chondrosarcoma is applied to a high-grade sarcoma occurring next to a low-grade malignant cartilage-forming tumour; it comprises approximately 10 per cent of all chondrosarcomas.[1] The tumour generally occurs after the age of 50 years, with males and females equally affected.[2] It is most often located in the bones of the pelvis, the proximal femur or humerus, the distal femur, and the ribs. Regardless of treatment, the prognosis is ominous with 90 per cent of patients dying with distant metastases within 2 years.[3]

Microscopically, the junction between the cartilaginous and the non-cartilaginous anaplastic component is remarkably sharp. The non-cartilaginous component may express features of a malignant fibrous histiocytoma (MFH), osteosarcoma, fibrosarcoma, rhabdomyosarcoma or angiosarcoma, with MFH features most frequently present. In 25 per cent of cases, the diagnosis is made at the time of local recurrence.[2] Distant metastases usually consist solely of the high-grade anaplastic component.

Controversy remains as to whether the anaplastic and cartilaginous components are derived from a common precursor cell,[4] or whether the anaplastic component represents a separate genotypic lineage (collision tumour).[5–7] We investigated both components of a case of dedifferentiated chondrosarcoma by loss of heterozygosity (LOH) analysis, comparative genomic hybridization (CGH), DNA flow cytometry, p53 immunohistochemistry, and p53 mutation analysis, in order further to elucidate the histogenesis of this rare entity.

MATERIALS AND METHODS

Clinical information

A 50-year-old female presented with a 6-month history of right sided hip complaints. Radiographs and CT scan revealed a large lytic lesion in the diaphysis of the proximal right femur, with intralesional calcifications and cortical thickening. MRI revealed intermediate signal intensity on the T1-weighted MR images and a high signal intensity on the T2-weighted MR images.

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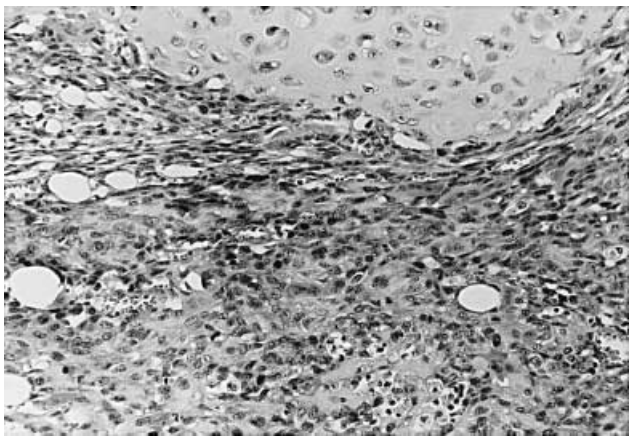


Fig. 1—Histological appearance of the primary resection specimen. Above, the moderately cellular cartilaginous component can be seen. The boundary between the two components is remarkably sharp. The anaplastic component showed a high-grade spindle cell tumour in which transformation to an osteosarcomatous phenotype was seen

Combined imaging features were therefore highly suggestive of a malignant cartilaginous tumour. A Jamshidi trocar biopsy was performed and histological examination revealed a chondrosarcoma grade II, according to Evans.[8] Subsequent treatment consisted of resection of the tumour with reconstruction by inlay allograft. The resected specimen showed at cut surface a grey white glassy tumour within the proximal femur, with a length of 8·5 cm. Routine sections demonstrated a moderately cellular chondroid tumour with binucleated cells and sporadic mitosis (grade II), next to a relatively small high-grade undifferentiated component in the proximal part of the resected specimen, reaching to the proximal osteotomy. This component showed a spindle cell phenotype and a high number of mitoses (up to 3 per HPF), in which focal transformation towards an osteosarcomatous phenotype was seen, with focal deposition of osteoid matrix surrounding the tumour cells. There was an abrupt margin between the cartilaginous and anaplastic areas (Fig. 1). The case was additionally evaluated by The Netherlands Committee on Bone Tumours and the diagnosis of dedifferentiated chondrosarcoma was supported after expert clinical-radiological and pathological review. Staging studies revealed no evidence of metastatic disease and adjuvant chemotherapy (adriamycin and cisplatin) was administered.

Six months later, the patient re-entered the clinic because of pain caused by tumour recurrence and a disarticulation of the right leg was performed. Histological examination revealed highly cellular undifferentiated tumour tissue with round-oval nuclei and scattered mitoses. CT scan demonstrated multiple lung metastases and the patient died 15 months after the first diagnosis.

Specimens

From the cartilaginous component, formalin-fixed, paraffin-embedded, and fresh frozen tumour tissue was obtained from the proximal femur resection. From the anaplastic component, formalin-fixed material was available. Fresh frozen tissue from this component was additionally derived from the amputation specimen.

DNA isolation

DNA isolation from fresh frozen tissue, with tumour percentages estimated on cryostat sections to be more than 80 per cent, was performed using proteinase K treatment and phenol–chloroform extraction as previously described,[9] with some modifications. Prior to proteinase K digestion, tumour tissue was pre-incubated in 3 M sodium acetate buffer, pH 5·6, saturated with hyaluronidase for 2 h at 37°C. The pH was then adjusted by adding 1/8 volume of 2 M sodium hydroxide. Normal DNA from a freshly collected blood sample of the same patient was isolated using a salting-out procedure.[10]

Loss-of-heterozygosity analysis

Analysis of microsatellite markers was performed by polymerase chain reaction (PCR) on 100 ng of DNA as described by Weber and May[11] using 1 μCi of [α^{32}P]dCTP in a total volume of 12 μl. Thermal cycling was performed in a programmable heatblock (MJ Research, Watertown, MA, U.S.A.) consisting of 27 cycles with an annealing temperature of 55°C. The microsatellite markers used were selected because they map to chromosome regions reported to be involved in chondrosarcoma. Apart from primers in the *EXT* and *EXT*-like region, primers were chosen in the p53[12] and Rb[12] region, at 9p21,[13] and at 10p15 and 10q11.[14] Polymorphic markers are described in the Genome Database (http://gdbwww.gdb.org). Chromosomes 4, 13, and 17 were investigated in detail using D4S230, D4S43, D4S189, D4S190, D4S1629, D4S231, D4S192, D4S175, D4S171, D4S194, ANT1, D13S175, D13S115, D13S217, FLT1, D13S220, D13S168, D13S124, D13S159, D13S173, D17S1532, D17S578, D17S520, D17S514, THRA1, D17S855, D17S1322, D17S1323, D17S1327, D17S579, D17S588, HOX2B, MPO, HGF, D17S1818, D17S1861, and D17S1692. After electrophoresis on a 6·5 per cent polyacrylamide gel containing 7 M urea, gels were dried and exposed to X-ray films. Signal intensities were measured by Phosphor Imaging (Molecular Dynamics, Sunyvale, CA, U.S.A.). LOH was scored when the quotient of the ratios of both alleles of normal and tumour was larger than or equal to 1·7.[15] Ratios between 1·3 and 1·7 were regarded as inconclusive.[16]

Comparative genomic hybridization

The CGH procedure was based on the protocol described by Kallioniemi *et al.*,[17] with a few modifications as described previously.[18] Briefly, test DNA was directly labelled with FITC-dUTP and reference DNA was labelled with lissamine-dUTP (NEN LifeSciences, duPont), both by nick translation. Nick-translated fragment sizes ranged from 400 to 2000 bp. Two hundred nanograms of each labelled DNA and 10 μg of Cot-1

Table I—List of the microsatellite markers tested. Mean allelic imbalance ratios are given for both the cartilaginous and the anaplastic components. Ratios above 1·7 were considered as loss of heterozygosity and are in bold. Inconclusive ratios are given in italics

Gene	Marker	Chromosomal localization	Hetero-zygosity	Cartilaginous component	Anaplastic component
EXT1	D8S85	8q23.3	0·74	1	1
	D8S547	8q24.11	0·66	n.i.	n.i.
	D8S522	8q24.12–13	0·71	n.i.	n.i.
	D8S198	8q24.13	0·83	1	1
EXT2	D11S905	11p13-p12	0·74	1·12	*1·54*
	D11S903	11p13-q13	0·74	1	1
	D11S554	11p11.2–12	0·91	1	**1·77**
EXT3	D19S216	19pter-qter	0·75	1·11	1·08
	D19S413	19pter-qter	0·76	n.i.	n.i.
	D19S221	19p13.2	0·86	1	1
EXTL1	D1S436	1p36	0·75	1·14	*1·33*
	D1S470	1p36	0·76	1·04	*1·48*
EXTL2	D1S206	1p11–12	0·82	1·06	1·28
	D1S248	1p11–12	0·82	n.i.	n.i.
EXTL3	D8S1130	8p12-p22	0·93	1·12	**3·3**
	gata119c	8p12-p21		n.i.	n.i.
	D8S1820	8p	0·73	1·08	**3·27**
	D8S283	8p	0·78	1·05	**2·92**
p53	TP 53	17p13.1	0·69	**4·69**↑	**2·91**↑
	D17S513	17p13.3	0·89	**7·6**↑	**4·8**↑
Rb	D13S153	13q14.1–14.3	0·82	**4·8**↑	**3·96**↑
	D13S155	13q14.3-q21.2	0·83	**3·55**↓	**2·02**↓
10p15	D10S559	10pter-p11.2	0·80	n.i.	n.i.
	D10S1435	10p	0·32	1·01	**3·3**
	D10S89	10p		n.i.	n.i.
10q11	D10S604	10pter-qter	0·66	n.i.	n.i.
	D10S538	10pter-qter	0·73	1·01	**2·03**
	D10S109	10q11.2-qter	0·71	1·16	**2·4**
	D10S110	10q11.2-qter	0·58	n.i.	n.i.
	D10S185	10q23-24	0·77	1·05	**2·01**
	D10S575	10q26	0·63	1·1	**2.1**
9p21	D9S43	9p21	0·83	1·1	
	D9S171	9p21	0·80	1·03	1·27

n.i.= non-informative (homozygous) at the locus; ↑=upper allele lost; ↓=lower allele lost.

DNA were hybridized to normal male metaphases and incubated at 37°C for 4 days. Post-hybridization washes were performed with 2 × SSC at 37°C, followed by 0·1 × SSC at 60°C. Slides were counterstained with DAPI in an antifade solution. Digital images were analysed using QUIPS XL software from Vysis (Downers Grove, IL, U.S.A.). Losses of DNA sequences were defined as chromosomal regions where the average green-to-red ratio and its 95 per cent confidence interval are below 0·9, and gains above 1·1. These threshold values were based on measurements from a series of normal controls.

DNA flow cytometry

Single cell suspensions from fresh frozen tissue for single-parameter nuclear DNA flow cytometry (FCM) were prepared by the method of Vindelov *et al.*[19] and measured on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, U.S.A.). Trout red blood cells served as an internal standard for determination of the G1 cell DNA content.[20] ModFitLT V2.0 software was used for data acquisition. DNA histograms were evaluated according to accepted criteria.[21]

Immunohistochemistry

Monoclonal antibodies directed to Ki-67, clone MIB1 (Immunotech SA, Marseilles, France) and p53, clone DO-7 (DAKO, Glostrup, Denmark) were used. Immunohistochemical reactions were performed on formalin-fixed, paraffin-embedded tumour sections according to standard laboratory methods.[22] As a negative control, slides were incubated with phosphate-buffered saline containing 1 per cent bovine serum albumin, instead of primary antibodies. Positive controls included a normal tonsil for Ki-67 and a p53-positive colorectal carcinoma for p53. Ki-67-positive nuclei were counted per 200 tumour cells, in areas containing the largest number of positive cells.

Table 2—Loss of heterozygosity analysis on chromosomes 4, 13, and 17 was performed using markers spanning the whole chromosome. Only heterozygous markers are shown. Allelic imbalance ratios are given for both the cartilaginous and the anaplastic component

Marker	Chromosomal localization	Cartilaginous component*	Anaplastic component*
D4S174	4p21.1-p14	**6·34↑**	**2·80↓**
D4S230	4pter-p15	**6·63↑**	**3·64↓**
D4S43	4p16.3	**6·27↑**	**2·94↓**
D4S190	4p21.1-p14	**2·19↑**	**1·77↓**
D4S1629	4pter-qter	**6·34↑**	**3·19↓**
D4S192	4q25-34	**5·82↑**	**3·50↓**
D4S175	4q31	**7·12↑**	**2·72↓**
ANT1	4q35	**3·63↓**	**2·90↑**
D13S115	13q11–12.1	**3·75↑**	**2·70↑**
FLT1	13q12	**5·47↑**	**3·37↑**
D13S153	13q14.1-q14.3	**4·8↑**	**3·96↑**
D13S155	13q14.3-q21.2	**2↓**	**2↓**
D13S168	13q14.3	**7·03↑**	**4·75↑**
D13S159	13q32	**6·99↓**	**2·98↓**
D17S513	17p13.3	**7·6↑**	**4·8↑**
TP 53	17p13.1	**4·69↑**	**2·91↑**
D17S520	17p12	**8·66↓**	**3·88↓**
THRA1	17q11.1-q12	**15·67↑**	**8·17↑**
D17S855	17q21	**1·77↓**	**4·19↑**
D17S588	17q	*1·69↑*	**3·57↓**

↑=upper allele lost; ↓=lower allele lost.
*Ratios above 1·7 were considered as LOH and are in bold. Inconclusive ratios are given in italics.

p53 mutation analysis

PCR for exons 5–8 of the p53 gene was performed on 100 ng of DNA in a total volume of 25 μl containing 12·5 pmol of the forward and 12·5 pmol of the reverse primer, 1 per cent BSA, 0·01 per cent gelatin, 0·1 per cent Triton X-100, 10 mM Tris–HCl (pH 9·0), 50 mM KCl, 1·5 mM $MgCl_2$, 0·2 mM dNTP, and 0·06 U of SuperTaq DNA polymerase (Sphaero Q, HT Biotechnology Ltd., Cambridge, U.K.). The primers used for amplification were: exon 5 forward: TTCCTCTTCCT GCAGTACTC, reverse: TCTCTGCTGTCCCGACC AAC; exon 6 forward: TGGGGCTGGAGAGACGAC, reverse: AGGGATATTGGGGTACTCTACAC; exon 7 forward: GTGTTATCTCCTAGGTTGGC, reverse: AGGTCCAGTCCTCGGTGAAC; exon 8 forward: TGATTTCCTTACTGCCTCTTG, reverse: CACGT-CAATACGGAGTCTAA. Thermal cycling was performed in a programmable heatblock (Perkin Elmer Cetus, Norwalk, CT, U.S.A.) using a 'touchdown' PCR program of 47 cycles consisting of two cycles with annealing at 65°C, two cycles at 64°C, two cycles at 63°C, two cycles at 62°C, two cycles at 61°C, two cycles at 60°C, four cycles at 59°C, six cycles at 58°C, and 25 cycles at 57°C. PCR products were purified using Micro-Spin G-50 columns (Pharmacia Biotech, Uppsala, Sweden). Subsequent sequencing reactions were performed using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, according to the manufacturer's recommendations (Perkin Elmer Applied Biosystems, Foster City, CA, U.S.A.). Samples were run on the ABI 377 semi-automated sequencer (Perkin Elmer, The Netherlands). Both strands were sequenced.

RESULTS

Loss of heterozygosity (LOH)

The cartilaginous component demonstrated LOH at 17p13, 13q14, and chromosome 4 (Tables I and II). The anaplastic component demonstrated loss of the same alleles on 17p13 and 13q14 and, additionally, LOH was detected on chromosomes 8, 10, and 11. LOH at chromosome 4 was also identified, but involved the other allele than in the cartilaginous component (Table II). This phenomenon involved all markers tested, spanning chromosome 4. For all markers spanning chromosome 13, the same allele was lost in both components. On

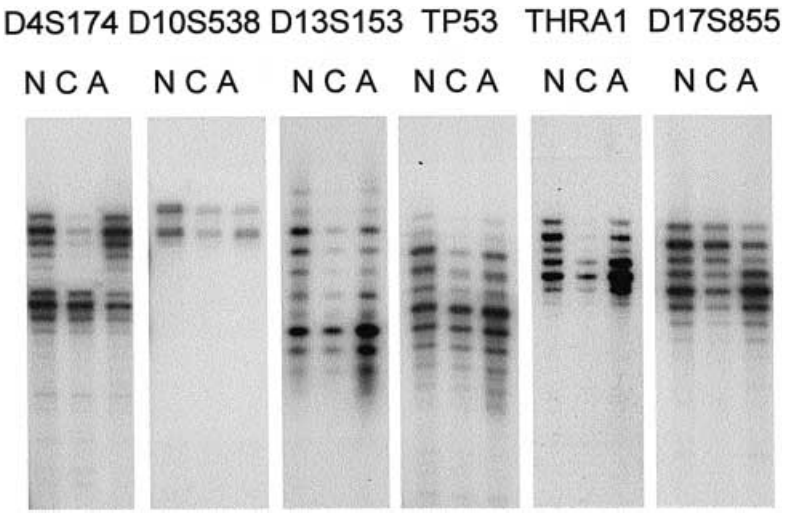


Fig. 2—Examples of autoradiograms. Microsatellite markers are indicated above each panel. N=normal DNA; C=cartilaginous component; A=anaplastic component. Allelic imbalance ratios are given in Tables I and II

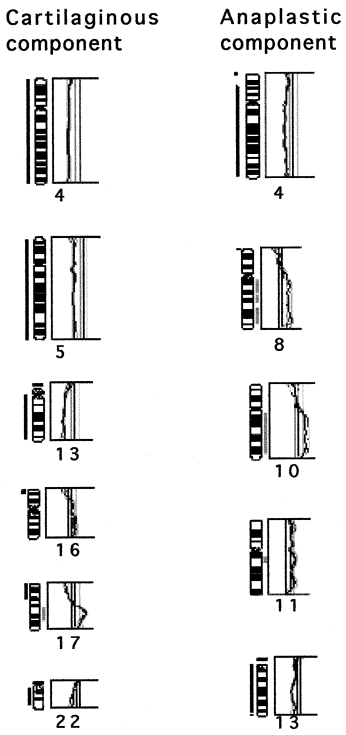


Fig. 3—Examples of unbalances found by CGH analyses in the cartilaginous and anaplastic components. The profiles show the average fluorescence intensity ratios and its 95 per cent confidence interval. Over- and under-representations are shown as grey bars on the right and black bars on the left sides of the ideograms, respectively.

chromosome 17, both components showed loss of the same allele for markers located on the short arm and the centromeric part of the long arm, whereas on the rest of the long arm, different alleles were lost in the two components, with relatively low allelic imbalance ratios in the cartilaginous component (Table II). Examples are shown in Fig. 2.

Comparative genomic hybridization

The cartilaginous component was characterized by the deletion of chromosomes 4, 5, 13, 22 and the distal part of chromosome 16p. Chromosome 17 revealed a deletion: del(17)(pter-q12) and an amplification on the long arm. In contrast, the anaplastic component demonstrated amplification on 2p, 8q, 10q, 11q, 12p, 12q, 19p, and possibly on 1p. Deletion was seen on 2q, 4, 5q, 13, Xp, and Xq (Fig. 3).

Flow cytometry

Flow cytometric analysis demonstrated two peri-diploid clones in the cartilaginous component, with DNA indices of 0·95 and 1·11. The anaplastic component revealed two highly aneuploid clones of 1·60 and 2·84 respectively (Fig. 4).

Immunohistochemistry

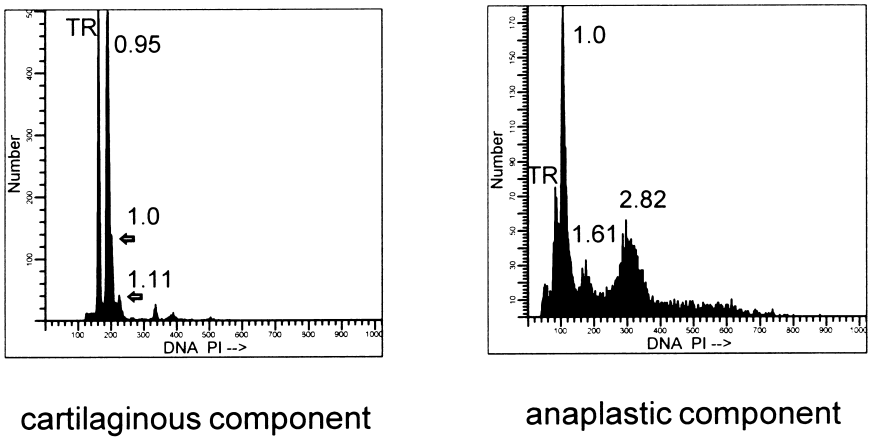
p53 immunoreactivity was seen in both components present in the primary tumour (Figs 5A and 5B), as well as in the anaplastic component of the recurrence. Proliferative activity in the primary tumour was higher in the anaplastic component than in the cartilaginous component (45 per cent versus 16 per cent Ki-67-positive cells), whereas in the recurrence specimen, 75 per cent positive cells were found.

p53 mutation analysis

Mutation analysis revealed a 6 bp deletion (CCATCC) starting at the second base of codon 250 in exon 7 in both the cartilaginous and the anaplastic component which was absent in normal DNA of the patient (Figs 5C and 5D). The deletion leads to the elimination of the amino acids proline and isoleucine from the central DNA-binding domain of the p53 protein.

DISCUSSION

In 1971, Dahlin and Beabout first described dedifferentiated chondrosarcoma as a rare variant within the



cartilaginous component anaplastic component

Fig. 4—Flow cytometric DNA histograms from nuclear suspensions of both the cartilaginous and the anaplastic component. Both demonstrate two different tumour cell clones, the cartilaginous component with peri-diploid DNA indices and the anaplastic component with highly aneuploid DNA indices.

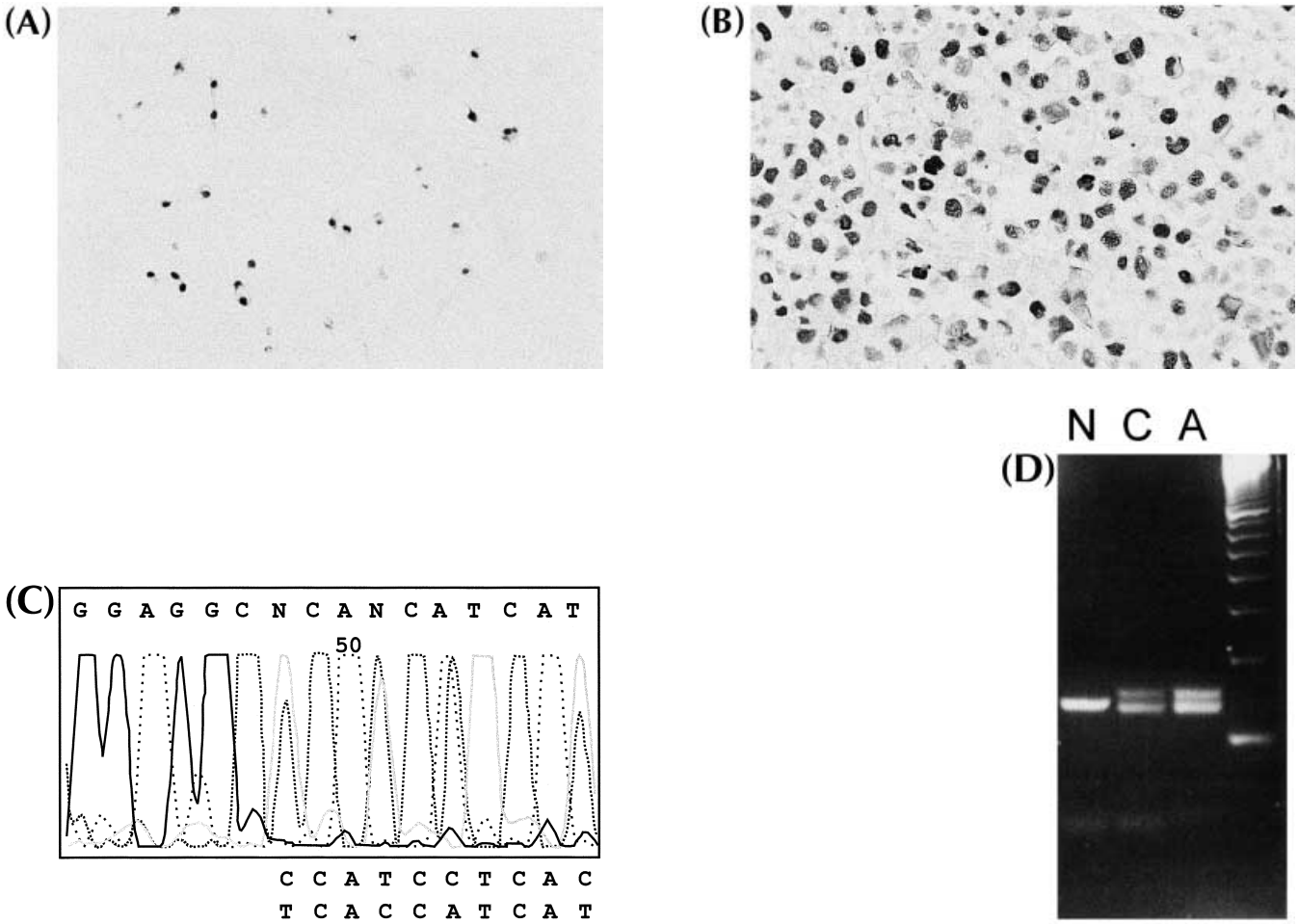


Fig. 5—p53 immunoreactivity was detected in both the cartilaginous (A) and the anaplastic (B) component. Sequencing results of exon 7 revealed a 6 bp deletion which was found in both components and not in DNA derived from peripheral blood (C). Starting at nucleotide 749, the second base of codon 250, two sequences running through each other can be detected and are indicated below. The upper sequence is the wild type, whereas the lower is the mutated sequence. PCR products of exon 7 were run on an agarose gel containing 3 per cent NuSieve GTG combined with 1 per cent Seakem GTG (both purchased from FMC BioProducts, Rockland, Maine, U.S.A.) (D). The 6 bp deletion was confirmed in both the cartilaginous and the anaplastic component and was not present in the germline

spectrum of malignant cartilaginous tumours. They explained the co-existence of the cartilaginous and anaplastic components as dedifferentiation of a well-differentiated cartilaginous tumour cell to a primitive undifferentiated tumour cell[23] (Fig. 6A). Sanerkin and Woods explained the presence of the anaplastic component as an independently developing sarcoma, arising within the reactive fibrous tissue surrounding necrotic areas in a pre-existing benign cartilaginous lesion.[7] Since both components would then arise within different tissues, dedifferentiated chondrosarcoma should be referred to as a 'collision' tumour (Fig. 6B). Currently, the generally supported hypothesis is that high-grade elements represent a failure of differentiation, rather than dedifferentiation of mature chondroid cells.[6,24] It is still debated as to whether the two components are derived from two separate clones of cells (collision tumour)[5,6] (Fig. 6B), or whether both components arise from a common primitive mesenchymal cell progenitor, possessing both the ability to differentiate and express chondrocytic features and the ability to express features of high-grade sarcomas[4] (Fig. 6C).

Phenotypic characteristics of both components have been studied intensively by electron microscopy and immunohistochemistry. Most ultrastructural studies revealed the lack of chondroid features in the anaplastic component.[6,25,26] Sparse S-100 protein-positive cells in the anaplastic component were interpreted as reflecting a retained potential for primitive chondrogenesis.[26,27] An immunohistochemical study for collagen subtypes and cartilage proteoglycans indicated a non-chondrocytic nature of the anaplastic component, favouring the 'collision tumour' theory.[28] Since these studies are based on phenotypically reversible features, they do not provide evidence for the histogenesis of dedifferentiated chondrosarcoma, in contrast to studies investigating irreversible, fixed molecular genetic alterations.

Comparative molecular genetic studies of both components of dedifferentiated chondrosarcoma are sparse. Cytogenetic analysis was performed in eight cases[4,29–33] and characteristic alterations have not been demon-

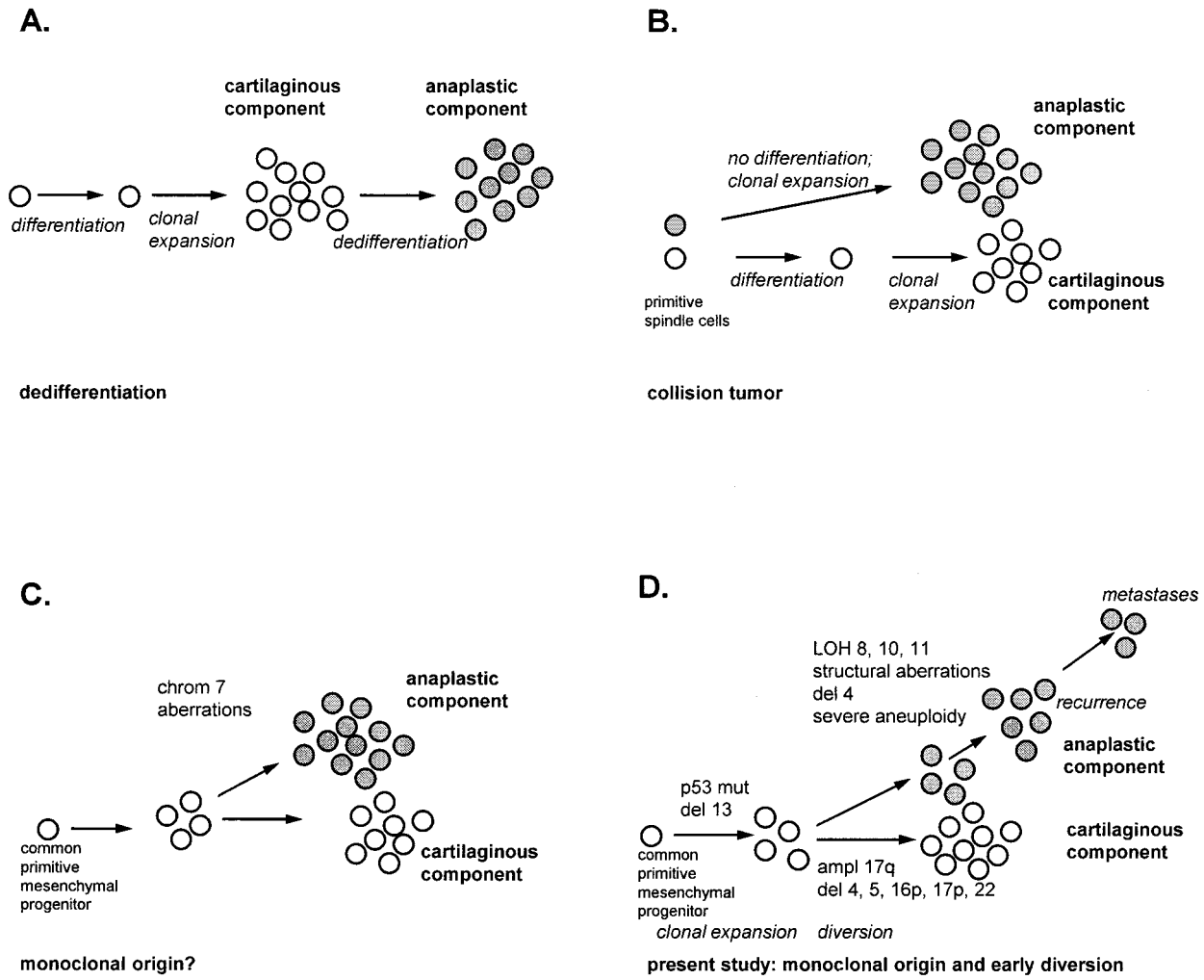


Fig. 6—Theories of the histogenesis of dedifferentiated chondrosarcoma. (A) Originally it was thought that a well-differentiated cartilaginous tumour cell dedifferentiates into a primitive undifferentiated tumour cell.[23] (B) The two components are derived from two separate clones of cells (collision tumour), one of which differentiates into a low-grade chondrosarcoma, while the other fails to differentiate and displays features of high-grade sarcoma.[5,6,28] (C) Both components of dedifferentiated chondrosarcoma display numerical aberrations of chromosome 7,[4] suggesting that both components are derived from a single abnormal clone or cell. (D) The present case provides compelling evidence for a monoclonal origin, since both components have specific genetic alterations in common. However, the presence of many additional different genetic alterations suggests that the separation of the two clones was a relatively early event

strated. Most karyotypes are probably derived from the anaplastic component, overgrowing the slowly dividing cartilaginous component in tissue culture. Bridge *et al.* were the first to combine cytogenetic analysis with immunophenotyping and showed numerical aberrations of chromosome 7 in both components of dedifferentiated chondrosarcoma,[4] which would suggest that both were derived from a single abnormal clone or cell. However, it cannot be completely ruled out that both components independently obtained an extra chromosome 7. Extra copies of chromosome 7 were seen in three other cases,[30,32,33] while CGH analysis of the present case did not show copy number changes of chromosome 7.

The results of the present case provide strong evidence to support a common origin. In both components, we identified an identical 6 bp deletion in p53 which was not reported in a p53 mutation database (http://p53.genome.ad.jp) and is therefore not regarded as a mutational hotspot. This provides strong evidence for a monoclonal origin and excludes a collision tumour in this case of dedifferentiated chondrosarcoma (Fig. 6D). Furthermore, LOH at 17p and 13 involved the same alleles.

However, we also found clear genetic differences. The anaplastic component showed LOH at additional loci that were not involved in the cartilaginous component (11p11.2–12, 8p, and chromosome 10), and amplification and deletion of several chromosome parts as demonstrated by CGH. In contrast, the cartilaginous component had lost chromosomes 5, 22, and the tip of 16p and revealed an amplification on 17q. Although at 17p both components lost the same alleles for all microsatellite markers tested, CGH analysis revealed a deletion of 17p in the cartilaginous component only. LOH analysis further revealed that both components

had lost a different copy of chromosome 4. Moreover, the DNA indices indicate that it is unlikely that simple polyploidization of the 0·95 and 1·11 clone of the cartilaginous component has occurred in the anaplastic component, with DNA indices 1·60 and 2·84. Thus, our data show that many genetic alterations have occurred after the diversion of the two components and therefore suggest that the separation of the two clones was a relatively early event in the histogenesis of this dedifferentiated chondrosarcoma (Fig. 6D). This early separation may explain why many studies on the phenotypically reversible features of the two components demonstrated very different phenotypes, leading to the conclusion that dedifferentiated chondrosarcoma is a collision tumour.[6,7,25,26,28] It remains unclear, however, whether (primitive) chondroid features are expressed before the divergence of the two components, supporting the original idea of 'dedifferentiation', or after the separation of the two lines of differentiation.

Since fresh-frozen tumour tissue of the anaplastic component was derived from the tumour recurrence specimen, we cannot completely rule out the possibility that some of the alterations detected in the anaplastic component are additional aberrations of the recurrence that occurred in the course of tumour progression. Unfortunately, DNA extracted from formalin-fixed, paraffin-embedded decalcified bone tumour tissue most often fails in PCR amplification and comparative genomic hybridization.

The p53 alteration being an early event in chondrosarcomas is in contrast with the literature, since p53 overexpression and mutation are mainly found in high-grade chondrosarcomas.[12,34,35] Previously, p53 overexpression was reported in four dedifferentiated chondrosarcomas in the anaplastic component,[34] whereas only focal weak positivity was noted in the cartilaginous areas. In another case, no immunoreactivity was seen in the cartilaginous component and in a fibrosarcomatous component, while a MFH-like portion demonstrated strong immunoreactivity. A missense mutation (Arg→Thr in codon 249 of exon 7) was only found in DNA from the p53-positive area and was absent in the p53-negative specimens,[36] suggesting that in this case, p53 was involved after the divergence of the two components. The presence of p53 overexpression and mutation in both components of the present case may be explained by the fact that the cartilaginous component was grade II according to Evans,[8] while most dedifferentiated chondrosarcomas reported display a grade I cartilaginous component, as originally defined.[23] Capanna *et al.* showed, however, that the cartilaginous component was of moderate to high histological grade in 25 of 46 (54 per cent) dedifferentiated chondrosarcomas.[3]

In conclusion, the molecular genetic characterization of this case of dedifferentiated chondrosarcoma provides strong evidence for a monoclonal origin, since both components share an identical p53 mutation and deletion of the same copies of chromosome 13. Many different genetic alterations were also demonstrated, which occurred after the diversion of the two components. These results suggest that the separation of the two clones may be an early event in the histogenesis of dedifferentiated chondrosarcoma.

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