SPANDIDOS PUBLICATIONS

Rb-loss is associated with high malignancy in chondrosarcoma

M. RÖPKE[1], C. BOLTZE[2], B. MEYER[2], H.W. NEUMANN[1], A. ROESSNER[2] and R. SCHNEIDER-STOCK[2]

Departments of [1]Orthopedic Surgery and [2]Pathology, Otto von Guericke University, Magdeburg, Germany

Received April 28, 2005; Accepted June 24, 2005

Abstract. Loss of function of the human retinoblastoma gene (Rb) is a frequent genetic abnormality in human malignancies and causes a disturbance in the cell cycle and loss of normal proliferation and differentiation. We studied the loss of heterozygosity (LOH) of the Rb gene in 31 formalin-fixed, paraffin-embedded cartilaginous tumors using polymerase chain reaction. The tumors were subdivided into 8 cases of dedifferentiated (DD) chondrosarcoma, 17 cases of conventional chondrosarcoma (nine grade 1, seven grade 2 and one grade 3), 4 enchondromas and 2 chondroblastomas. Both components of DD chondrosarcoma, the low-grade and anaplastic components, were separated by a microdissection approach. The genetic data were correlated with the expression of the Rb protein examined by Rb immunohistochemistry. We found Rb-LOH in one grade 3 chondrosarcoma, and in the anaplastic component in 7 of 8 cases of DD chondrosarcoma (89% of all high-grade chondrosarcomas). All tumors with Rb-LOH were immunohistochemically Rb-negative. The only case of DD chondrosarcoma negative for Rb-LOH in both components of the tumor also showed weak expression of the Rb protein in the anaplastic component. All benign cartilaginous tumors, low-grade chondrosarcomas and low-grade tumor components of DD chondrosarcomas were negative regarding Rb-LOH but positive in Rb immunohistostaining. We concluded that Rb-LOH predominantly occurs in high-grade chondrosarcomas. However, it is not a marker for identifying low-grade tumors with a tendency towards progression or local recurrence.

Introduction

Loss of function of the human retinoblastoma gene (Rb) is an essential step in tumorigenesis (1). Uncontrolled cell proliferation is one of the hallmarks of cancer, and it is typical of tumor cells to contain damaged genes that directly regulate their cell cycles (2). Alterations in the Rb pathway and defects of the Rb gene and Rb protein are associated with the pathogenesis and development of several malignant tumors (3). It has been demonstrated that the loss of heterozygosity (LOH) and DNA alterations of the Rb gene indicate poor prognosis in osteosarcomas and soft tissue sarcomas (4-7). This also applies to chondrosarcoma, which is known to display alterations in p16, pRb and cdk4 at the protein and DNA levels (8-11).

Chondrosarcoma, the second most common primary malignant bone tumor, is characterized by a wide spectrum of clinical behavior patterns and graded with a clinicopathological system (12). However, the correlation of histological appearance with prognosis is poor for the individual cartilaginous tumor, especially in low-grade chondrosarcomas. Chondrosarcoma primarily arises in bone (primary chondrosarcoma) or originates from benign cartilaginous lesions, such as osteochondroma or enchondroma (secondary chondrosarcoma). Further research is necessary to elucidate the process of tumor progression from a low-grade cartilaginous tumor to a high-grade chondrosarcoma with poor prognosis.

The pathogenesis of the so-called dedifferentiated (DD) chondrosarcoma still needs to be clarified. This special entity of a high-grade malignant tumor contains two different histological compartments. One component morphologically resembles enchondroma or a conventionally low-grade chondrosarcoma, and the other contains a variant of a high-grade mesenchymal neoplasm, e.g. malignant fibrous histiocytoma, fibrosarcoma, osteosarcoma, rhabdomyosarcoma or angiosarcoma (13).

Rb protein phosphorylation triggered by cyclin D-dependent kinases is essential for passage at the G1 restriction point and entry into S phase (2). The hypophosphorylated Rb protein binds to E2F, a transcriptional regulator. Phosphorylation of the Rb protein releases E2F, which transactivates genes whose products are important for cell-cycle progression. These central functions of the Rb tumor suppressor protein explain its role in cell proliferation and tumor progression.

Previous studies have shown the significance of Rb-LOH in chondrosarcomas (11). Furthermore, it has been reported that Rb protein plays an essential role in chondrocyte proliferation, differentiation and growth arrest during enchondral bone development (14,15). Therefore, the aim of our study was to clarify whether alterations occur during the transition from benign to low-grade or from low-grade to high-grade malignant cartilaginous tumors. In this respect, it is interesting to investigate each component of DD chondrosarcomas separately, both the low-grade cartilaginous and high-grade anaplastic parts. To clearly separate the samples

Correspondence to: Dr Regine Schneider-Stock, Department of Pathology, Otto-von-Guericke University, Leipziger Strasse 44, D-39120 Magdeburg, Germany
E-mail: regine.schneider-stock@medizin.uni-magdeburg.de

Key words: Rb-LOH, retinoblastoma gene, loss of heterozygosity, dedifferentiated chondrosarcoma, tumour progression, microdissection

Table I. Histopathological and genetic data of dedifferentiated chondrosarcomas.

ID no.	Age	Sex	Localization	Tumor type	Histological subtype	Rb-LOH	Rb-IH
1	74	M	Femur	P	Low grade	-	+++
					Anaplastic (OS)	+	-
2	68	M	Femur	P	Low grade	-	+++
					Anaplastic (FS)	+	-
3	49	M	Sacrum	P	Low grade	-	+++
					Anaplastic (MFH)	+	-
4	61	F	Femur	P	Low grade	-	+++
					Anaplastic (OS)	+	-
5	77	F	Femur	P	Low grade	-	+++
					Anaplastic (MFH)	+	-
6	68	F	Femur	R	Low grade	-	+++
					Anaplastic (OS)	+	-
7	63	M	Femur	P	Low grade	-	+++
					Anaplastic (MFH)	+	-
8	64	M	Vertebra	P	Low grade	-	+++
					Anaplastic (MFH)	-	+

OS, osteosarcomatous; FS, fibrosarcomatous; MFH, malignant fibrous sarcoma; P, primary tumor; R, local recurrence; M, male; F, female. Rb nuclear immunoreactivity: -, <30%; +, 30-50%; ++, 50-80%; +++, >80%.

Table II. Histopathological and genetic data of cartilaginous tumors.

ID no.	Age	Sex	Localization	Tumor type	Histological subtype	Rb-LOH	Rb-IH
9	34	F	Humerus	P	CS I	-	+++
10	42	M	Humerus	P	CS I	-	+++
11	71	M	Humerus	P	CS I	-	+++
12	28	F	Ilium	P	CS I	-	+++
13	73	F	Scapula	R	CS I	-	+++
14	78	F	Rib	P	CS I	-	+++
15	61	F	Vertebra	P	CS I	-	+++
16	74	F	Tarsus	R	CS I	-	+++
17	34	M	Tibia	P	CS I	-	+++
18	49	M	Femur	P	CS II	-	+++
19	19	M	Tibia	P	CS II	-	+++
20	64	F	Humerus	P	CS II	-	+++
21	69	M	Femur	P	CS II	-	++
22	35	M	Tibia	P	CS II	-	+++
23	62	M	Ilium	P	CS II	-	++
24	46	M	Pelvis	R	CS II	-	+++
25	38	M	Humerus	R	CS III	+	-
26	46	F	Tibia	P	EC	-	+++
27	31	F	Tibia	P	EC	-	+++
28	56	M	Tibia	P	EC	-	+++
29	48	F	Femur	P	EC	-	+++
30	9	F	Humerus	R	CB	-	+++
31	27	F	Humerus	P	CB	-	+++

CS, chondrosarcoma; I-III, grade of malignancy; EC, enchondroma; CB, chondroblastoma; P, primary tumor; R, local recurrence; M, male; F, female. Rb nuclear immunoreactivity: -, <30%; +, 30-50%; ++, 50-80%; +++, >80%.

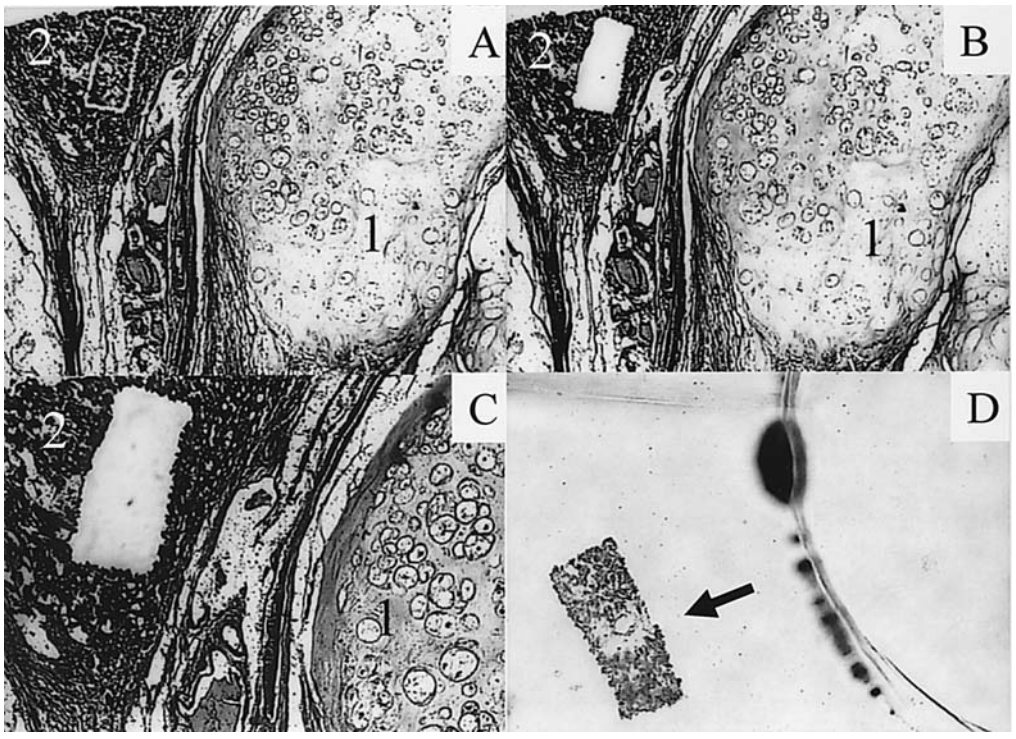

Figure 1. Overview of laser microdissection process. (A) H&E staining of the well-differentiated cartilaginous part (1) and anaplastic focus (2) of the dedifferentiated chondrosarcoma; the microdissected area is visible in the anaplastic part (2); a single laser shot ejected the microdissected sample from the object slide (B and C) and catapulted the tissue directly into the cap of a PCR tube (D).

from each other, we used the laser microdissection approach. In addition, we examined chondroblastomas and enchondromas as well as chondrosarcomas of different grades of malignancy.

Materials and methods

Tumors. We investigated formalin-fixed, paraffin-embedded tissue blocks obtained from a total of 31 chondrogenic tumors (8 DD chondrosarcomas, 17 conventional chondrosarcomas and 6 benign cartilaginous tumors). The clinicopathological data are summarized in Tables I (DD chondrosarcomas) and II (cartilaginous tumors). All cases were collected from the histopathological files of the Department of Pathology, Otto von Guericke University, Magdeburg, and sent from the Department of Orthopedics of the Otto von Guericke University, Magdeburg, or from other remote institutions for histopathological assessment or consultation. In 13 cases, long-term follow-up data were available.

DNA isolation. Chondroblastic and dedifferentiated regions, as well as non-neoplastic reference tissue, were selected from formalin-fixed, paraffin-embedded sections using macrodissection with a razor blade or, if necessary, by laser microdissection (PALM) (Fig. 1). DNA was prepared according to the manufacturer's instructions (Machery and Nagel, Germany).

Rb-LOH. LOH analysis was performed as described elsewhere (16). Briefly, three intragenic Rb markers [two with restriction fragment-length polymorphism (RFLP) at introns 1 and 25, and one with variable-number tandem repeat (VNTR) polymorphism at intron 20] were analyzed using PCR. Primer sequences were: for Rb intron 1, sense 5'-CAGGAC AGCGGCCCGGAG-3' and antisense 5'-CTGCAGACGCTC CGCCGT-3'; for Rb intron 20, sense: 5'-TGAGCACCCAGA ATTAGAAC-3' and antisense 5'-TTAACAAGGTGTGGT GGTAC-3'; and for Rb intron 25, sense 5'-TCCATTTAT AAATACACATG-3' and antisense 5'-TAACGAAAAGAC TTCTTGCA-3'. Rb markers were amplified as follows: 95˚C for 5 min, 20 cycles at 95˚C for 10 sec, 66˚C for 10 sec, and 72˚C for 20 sec, with a stepwise decrease of 2˚C at each fifth cycle, and finally 30 cycles at 95˚C for 45 sec, 58˚C for 1 min, and 72˚C for 1 min. We added 100 ng of DNA, 20 mM Tris-HCl, 1.5 mM $MgCl_2$, 25 pmol of each primer, 160 μM each dNTP and 0.2 units of Taq-polymerase (Gibco BRL) in a total reaction volume of 50 μl.

Restriction fragment analyses were performed using the endonucleases: *Bam*Hl for Rb intron 1 and *Dra*I for Rb intron 25 (AGS, Heidelberg, Germany). PCR fragments were run on native 8% polyacrylamide gels, cross-linked with piperazine diacrylamide, and visualized by silver staining (17).

Allelic loss was considered when, in comparison with non-neoplastic reference DNA, the signal of a tumor band disappeared, or signal intensity was reduced by >50%. Evaluation was made twice, visually, or by densitometry (VDS, Pharmacia Biotech) in ambiguous cases. If only one band was detectable in the non-tumor material, the case was regarded as non-informative.

Rb immunohistochemistry. Immunohistochemical studies were performed on representative formalin-fixed, paraffin-embedded material with the use of appropriate positive and negative controls throughout. Sections (4 μm thick) were deparaffinized in xylol and washed in distilled water,

ID-Nr.	Histo-pathology	Rb-LOH in Intron		
		1	20	25
1	low-grade		n.a.	
	anaplastic		n.a.	
2	low-grade		n.a.	
	anaplastic		n.a.	
3	low-grade		n.a.	
	anaplastic		n.a.	
4	low-grade	n.a.		
	anaplastic	n.a.		
5	low-grade		n.a.	
	anaplastic		n.a.	
6	low-grade	n.a.	n.a.	
	anaplastic	n.a.	n.a.	
7	low-grade	n.a.	n.a.	
	anaplastic	n.a.	n.a.	
8	low-grade			
	anaplastic		n.a.	

Figure 2. Detailed data of the Rb-LOH analysis in dedifferentiated chondrosarcomas. Note the partial Rb-loss in cases 1 and 2: no LOH for intron 1, but LOH in the 3' region of the Rb gene (intron 25). LOH ($_n$); heterozygous without LOH (grey box); non-informative ($_q$); n.a., not amplifiable.

followed by treatment in two heating steps: the first for 5 min at 650 W with Glyca buffer (1:10, pH 3.0; BioGenex), and the second for 15 min at 450 W. After cooling the sections to room temperature and rinsing with Tris buffer, the slides were incubated for 1 h at 37˚C with antibody anti-retinoblastoma protein (Rb1, polyclonal, 1:15; BioGenex) in the given dilution. For subsequent staining, we applied the ABC (avidin-biotin complex) method using the alkaline phosphatase technique for anti-Rb1 (Universal LSAB Plus kit AP; Dako, Hamburg, Germany). Nuclear counterstaining was carried out using hemalaun.

Results

PCR analysis. Informativity was highest for the intron 25 marker (20 total; 65%). In some cases, we failed to amplify the intron 1 and intron 20 markers because of the non-sufficient amount or poor quality of extracted DNA.

LOH at the Rb locus was detected in the anaplastic component in 7 of 8 cases of DD chondrosarcoma (Table I), and the only case of grade 3 chondrosarcoma (Table II). Partial loss was found in two cases (see ID nos. 1 and 2 in Fig. 2), but could not be excluded in the other cases. The microdissected low grade cartilaginous components of DD chondrosarcomas never showed Rb-LOH (Fig. 2). One DD chondrosarcoma had no Rb-LOH (ID no. 8) in the dedifferentiated MFH-like component. However, it is possible that a partial loss, as demonstrated in other cases, might be a reason for not finding Rb-LOH. In our study, no benign cartilaginous lesions or chondrosarcomas of grades 1 and 2 showed Rb-LOH at the Rb locus (Table II).

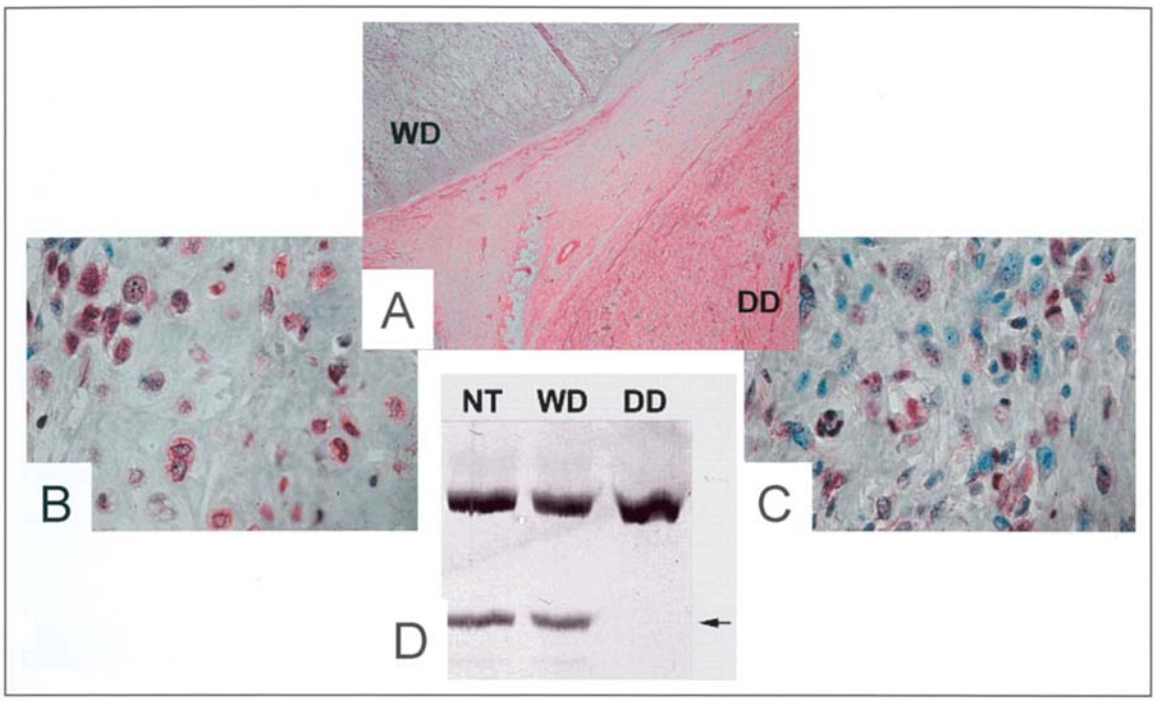

Figure 3. Rb immunohistochemistry in a dedifferentiated chondrosarcoma. (A) H&E staining of a dedifferentiated chondrosarcoma (ID no. 3, Table I) with the well-differentiated cartilaginous (WD) and anaplastic dedifferentiated (DD) parts; the well-differentiated part (B) showed strong nuclear positivity in >80% of tumour cells, whereas the anaplastic part (C) showed only 30% of tumour cells bearing a positive Rb immunoreaction. (D) Rb-LOH (←) for intron 25 in the anaplastic, dedifferentiated part of the dedifferentiated chondrosarcoma. NT, normal tissue.

Clinical history.

ID no.	Age/ sex	Localization	Histology	Clinical history	Surgical procedure of the observed CS	Outcome	Time of NED
3	49/M	Sacrum, periosteal dorsal	CS DD	Primary	Wide resection including partial resection of sacrum and musculocutaneous turnover flap	No recurrence	3 years
4	61/F	Proximal femur	CS DD	Primary	Wide resection	Died of a pulmonary embolism	2 months
6	68/F	Proximal femur	CS DD	Resection of a CS 6 years ago and reconstruction with arthoprosthesis, local recurrence with dedifferentiation	Wide resection	No recurrence	8 years
9	34/F	Proximal humerus	CS I	Primary	Wide resection and reconstruction using fibular autograft	No recurrence	6 years
10	42/M	Proximal humerus	CS I	Primary	Wide resection and substitution with arthroprosthesis	No recurrence	4 years
12	28/F	Ilium, ventral	CS I	Multiple hereditary exostoses primary	Wedge resection of the ilium	No recurrence	5 years
13	73/F	Scapula, ang. inferior	CS I	CS of the scapula, partial resection (ala scapulae) 5 years ago, local recurrence	Total resection of the scapula	No recurrence	4 years
15	61/F	3rd lumbar vertebra	CS I	Primary	Vertebral body implant after total resection	Further CS (grade I) of the 7th rib, resection of the rib, no recurrence	5 years
17	34/M	Proximal	CS I	Primary	Partial resection of the tibia	Local recurrence local resection, outcome unclear	Unclear
21	69/M	Proximal femur	CS II	Primary	Wide resection and substitution with arthroprosthesis	Local recurrence of the hip, hemipelvectomy	3 years
22	35/M	Proximal tibia	CS II	Ollier's disease, primary	Partial resection of the tibia	Local recurrence, wide resection and substitution with arthroprosthesis	2 years
24	46/M	Pelvis, hip, femoral neck	CS II	CS of the ilium, partial resection of the ilium 7 years ago	Hemipelvectomy	No recurrence	4 years
25	38/M	Proximal humerus	CS III	Multiple hereditary exostoses, resection of an exostosis 4 years ago in this region, local recurrence	Amputation thoraco-scapular	No recurrence	3 years

CS, chondrosarcoma; I-III, grade of malignancy; DD, dedifferentiated; EC, enchondroma; CB, chondroblastoma; NED, no evidence of disease.

Rb immunohistochemistry. All tissue samples were analyzed for expression of the Rb protein. All tumors showing Rb-LOH were immunohistochemically negative (Tables I and II, and Fig. 3). In the only case of Rb-LOH-negative DD chondrosarcoma, the Rb protein was weakly expressed in the anaplastic component of this tumor. Whereas all cases of grade 1 chondrosarcoma and most cases of grade 2 chondrosarcoma showed Rb protein expression of >50%, two grade 2 chondrosarcomas were <50%.

In addition, we have the results of Rb immunohistochemistry in five cases of failed PCR analysis. One case of enchondroma, one case of grade 1 chondrosarcoma and the low-grade component of one DD chondrosarcoma showed Rb protein expression >50%. However, the high-grade component of the one DD chondrosarcoma (<30%) and two grade 3 chondrosarcomas (both <30%) were immunohistochemically Rb-negative.

Clinical history of interesting cases with chondrosarcoma. A clinical history was available for only a few patients, as some cases were sent from institutions not related to our Department of Orthopedics. The complete clinical histories and follow-up data of 13 cases are presented in Table III. Because of this low number, a correlation with genetic or immunohistochemical data was impossible.

After wide or radical resection of the tumor, we found recurrences or metastases in none of the grade 3 or DD chondrosarcomas. Some cases of low-grade chondrosarcoma showing local recurrence were treated with resection, which led to better functional results. However, in these cases, surgical therapy did not correspond to the criteria of wide or radical resection. None of the patients died of disease in this group and one patient under postoperative radiation therapy died from pulmonary embolism.

Discussion

Defects in the cell-cycle regulatory pathways play an important role in the oncogenesis of chondrosarcoma. In our study, we found Rb-LOH in 89% of high-grade chondrosarcomas and DD chondrosarcomas. However, benign cartilaginous tumors and low-grade chondrosarcomas never showed Rb-LOH, an observation that concerns grade 1 or 2 chondrosarcomas, or the low-grade chondromatous component of DD chondrosarcomas. Therefore, we presume that the occurrence of Rb-LOH is significantly correlated with high malignancy.

In a previous study, Yamaguchi *et al* found Rb-LOH in 36% of a total 28 cases of chondrosarcoma with different histological grading (11). Grade 1 chondrosarcomas showed Rb-LOH in 18%, and grade 2 chondrosarcomas displayed Rb-LOH in 56%. The only case of grade 3 chondrosarcoma in that study, however, had no Rb-LOH. In 4 DD chondrosarcomas, Rb-LOH was found in 50% of cases (11). Unfortunately, samples of the high-grade and low-grade parts of DD chondrosarcomas were not examined separately in their study.

In contrast, Pompetti *et al* reported on alterations of Rb, p53, c-myc, N-myc, Ras and c-fos in bone tumors (osteosarcoma, giant cell tumor and chondrosarcoma) and found no Rb alterations in 18 chondrosarcomas using Southern, Northern or Western blotting analyses (18). Rb-LOH was neither found in a clear cell chondrosarcoma nor in two chondrosarcomas of the skull base (19,20). Bovee *et al* investigated the difference between peripheral and central chondrosarcomas and detected Rb-LOH in 71% of 17 peripheral and 12% of 12 central chondrosarcomas. All of the peripheral chondrosarcomas developed secondary to benign lesions (pre-existing osteochondroma) but only a third of all central chondrosarcomas were secondary tumors (21).

What might be the reason for the divergence of results of the above-mentioned papers? To clarify this problem, we tried to obtain clearly defined tissue samples for DNA preparation and used laser microdissection for separating the tumors, especially with regard to DD chondrosarcomas. The Rb gene located at chromosome 13 (13q14.2) is composed of 27 exons and 26 introns (22,23). To obtain more detailed information per case, we used three different polymorphic markers (intron 1, intron 20 and intron 25) for PCR analysis, as described by Schneider-Stock *et al* (6). In addition, we immunohistologically detected expression of the Rb protein and correlated these findings with the genetic results to understand the functional relevance of Rb-LOH. The combination of these methods should minimize possible errors of LOH analysis.

In normal chondrocytes, the Rb protein is involved in proliferation, differentiation and growth arrest (14,15). In accordance with other studies dealing with Rb-LOH in chondrosarcomas (9-11), we presume that Rb alterations occur in the transition from benign cartilaginous tumors to low-grade chondrosarcomas. In contrast, our results showed that Rb-LOH occurred neither in grade 1 or 2 chondrosarcomas nor in enchondromas and chondroblastomas. Therefore, Rb-LOH cannot be regarded as an early marker of this transition. We believe that Rb-LOH is an event affecting high-grade chondrosarcomas, as described for other sarcomas, such as osteosarcoma or liposarcoma (4-6). Alterations of tumor suppressor genes or oncogenes, such as p53 (24-26), H-ras (27) or p16 (8,9), are mainly found in high-grade chondrosarcomas. As an early event, they seem to be rather rare (10). Other authors showed that epigenetic alterations occur earlier in the development of chondrosarcomas (8,28,29). Further examination with a higher number of cases is necessary to support these results.

Although we regard the knowledge that Rb-LOH is a genetic event in high-grade chondrosarcoma to be essential, it seems to only be of low relevance to clinical practice. Rb-LOH does not have the potential to identify low-grade tumors with a tendency towards progression or local recurrence. However, the higher grade of malignancy can be demonstrated by Rb-LOH in unclear cases. In this study, the clinical history, as well as the follow-up data of some cases, demonstrated that surgical therapy is of special relevance. Compromises in surgical therapies favoring functionality often lead to local recurrence, and there is a high risk of administering inadequate therapies, particularly in cases of low-grade chondrosarcoma. Radical surgery will still act as the guarantor for the successful treatment of chondrosarcoma, and the evaluation of prognostic factors is possible only by correlation with surgical therapy.

1. Hong FD, Huang HJ, To H, Young LJ, Oro A, Bookstein R, Lee EY and Lee WH: Structure of the human retinoblastoma gene. Proc Natl Acad Sci USA 86: 5502-5506, 1989.
2. Sherr CJ: Cancer cell cycles. Science 274: 1672-1677, 1996.
3. Macleod K: Tumor suppressor genes. Curr Opin Genet Dev 10: 81-93, 2000.
4. Feugeas O, Guriec N, Babin-Boilletot A, Marcellin L, Simon P, Babin S, Thyss A, Hofman P, Terrier P, Kalifa C, Patricot LM, Brunat-Mentigny M and Oberling F: Loss of heterozygosity of the RB gene is a poor prognostic factor in patients with osteosarcoma. J Clin Oncol 14: 467-472, 1996.
5. Patino-Garcia A, Pineiro ES, Diez MZ, Iturriagagoitia LG, Klussmann FA and Ariznabarreta LS: Genetic and epigenetic alterations of the cell cycle regulators and tumor suppressor genes in pediatric osteosarcomas. J Pediatr Hematol Oncol 25: 362-367, 2003.
6. Schneider-Stock R, Boltze C, Jaeger V, Stumm M, Seiler C, Rys J, Schutze K and Roessner A: Significance of loss of heterozygosity of the RB1 gene during tumour progression in well-differentiated liposarcomas. J Pathol 197: 654-660, 2002.
7. Wurl P, Meye A, Berger D, Lautenschlager C, Bache M, Holzhausen HJ, Schmidt H, Dralle H, Rath FW and Taubert H: Significance of retinoblastoma and Mdm2 gene expression as prognostic markers for soft tissue sarcoma. Langenbecks Arch Surg 383: 99-103, 1998.
8. Asp J, Sangiorgi L, Inerot SE, Lindahl A, Molendini L, Benassi MS and Picci P: Changes of the P16 gene, but not the P53 gene in human chondrosarcoma tissues. Int J Cancer 85: 782-786, 2000.
9. Asp J, Inerot, S, Block JA and Lindahl A: Alterations in the regulatory pathway involving P16, PRb and Cdk4 in human chondrosarcoma. J Orthop Res 19: 149-154, 2001.
10. Bovee JV, Cleton-Jansen AM, Rosenberg C, Taminiau AH, Cornelisse CJ and Hogendoorn PC: Molecular genetic characterization of both components of a dedifferentiated chondrosarcoma, with implications for its histogenesis. J Pathol 189: 454-462, 1999.
11. Yamaguchi T, Toguchida J, Wadayama B, Kanoe H, Nakayama T, Ishizaki K, Ikenaga M, Kotoura Y and Sasaki MS: Loss of heterozygosity and tumor suppressor gene mutations in chondrosarcomas. Anticancer Res 16: 2009-2015, 1996.
12. Gitelis S, Bertoni F, Picci P and Campanacci M: Chondrosarcoma of bone. The experience at the Istituto Ortopedico Rizzoli. J Bone Joint Surg Am 63: 1248-1257, 1981.
13. Aigner T: Towards a new understanding and classification of chondrogenic neoplasias of the skeleton biochemistry and cell biology of chondrosarcoma and its variants. Virchows Arch 441: 219-230, 2002.
14. Dailey L, Laplantine E, Priore R and Basilico CA: Network of transcriptional and signaling events is activated by FGF to induce chondrocyte growth arrest and differentiation. J Cell Biol 161: 1053-1066, 2003.
15. Rossi F, MacLean HE, Yuan W, Francis RO, Semenova E, Lin CS, Kronenberg HM and Cobrinik D: P107 and P130 coordinately regulate proliferation, Cbfa1 expression, and hypertrophic differentiation during endochondral bone development. Dev Biol 247: 271-285, 2002.
16. Schneider-Stock R, Walter H, Radig K, Rys J, Bosse A, Kuhnen C, Hoang-Vu C and Roessner A: MDM2 amplification and loss of heterozygosity at Rb and P53 genes: no simultaneous alterations in the oncogenesis of liposarcomas. J Cancer Res Clin Oncol 124: 532-540, 1998.
17. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ and Allen RC: Analysis of the VNTR locus D1S80 by the PCR followed by high resolution PAGE. Am J Hum Genet 48: 137-144, 1991.
18. Pompetti F, Rizzo P, Simon RM, Freidlin B, Mew DJ, Pass HI, Picci P, Levine AS and Carbone M: Oncogene alterations in primary, recurrent, and metastatic human bone tumors. J Cell Biochem 63: 37-50, 1996.
19. Eisenberg MB, Woloschak M, Sen C and Wolfe D: Loss of heterozygosity in the retinoblastoma tumor suppressor gene in skull base chordomas and chondrosarcomas. Surg Neurol 47: 156-160, 1997.
20. Kleist B, Poetsch M, Lang C, Bankau A, Lorenz G, Jundt G, Suess-Fridrich K and Wolf E: Clear cell chondrosarcoma of the larynx: a case report of a rare histologic variant in an uncommon localization. Am J Surg Pathol 26: 386-392, 2002.
21. Bovee JV, Cleton-Jansen AM, Kuipers-Dijkshoorn NJ, Taminiau AH, van den Broek LJ, Cornelisse CJ and Hogendoorn PC: Loss of heterozygosity and DNA ploidy point to a diverging genetic mechanism in the origin of peripheral and central chondrosarcoma. Genes Chromosomes Cancer 26: 237-246, 1999.
22. Lee WH, Shew JY, Hong FD, Sery TW, Donoso LA, Young LJ, Bookstein R and Lee EY: The retinoblastoma susceptibility gene encodes a nuclear phosphoprotein associated with DNA binding activity. Nature 329: 642-645, 1987.
23. Lee WH, Bookstein R, Hong F, Young LJ, Shew JY and Lee EY: Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science 235: 1394-1399, 1987.
24. Coughlan B, Feliz A, Ishida T, Czerniak B and Dorfman HD: P53 expression and DNA ploidy of cartilage lesions. Hum Pathol 26: 620-624, 1995.
25. Dobashi Y, Sugimura H, Sato A, Hirabayashi T, Kanda H, Kitagawa T, Kawaguchi N, Imamura T and Machinami R: Possible association of P53 overexpression and mutation with high-grade chondrosarcoma. Diagn Mol Pathol 2: 257-263, 1993.
26. Wadayama B, Toguchida J, Yamaguchi T, Sasaki MS and Yamamuro T: P53 expression and its relationship to DNA alterations in bone and soft tissue sarcomas. Br J Cancer 68: 1134-1139, 1993.
27. Sakamoto A, Oda Y, Adachi T, Oshiro Y, Tamiya S, Tanaka K, Matsuda S, Iwamoto Y and Tsuneyoshi M: H-Ras oncogene mutation in dedifferentiated chondrosarcoma: polymerase chain reaction-restriction fragment length polymorphism analysis. Mod Pathol 14: 343-349, 2001.
28. Asp J, Brantsing C, Benassi MS, Inerot S, Sangiorgi L, Picci P and Lindahl A: Changes in P14 (ARF) do not play a primary role in human chondrosarcoma tissues. Int J Cancer 93: 703-705, 2001.
29. Ropke M, Boltze C, Neumann HW, Roessner A and Schneider-Stock R: Genetic and epigenetic alterations in tumor progression in a dedifferentiated chondrosarcoma. Pathol Res Pract 199: 437-444, 2003.