

Genome-wide Analysis of Sixteen Chordomas by Comparative Genomic Hybridization and Cytogenetics of the First Human Chordoma Cell Line, U-CHI

Stefanie Scheil,^{1*} Silke Brüderlein,¹ Thomas Liehr,² Heike Starke,² Jochen Herms,³ Michael Schulte,⁴ and Peter Möller¹

¹Institute of Pathology, University Hospitals of Ulm, Ulm, Germany

²Institute of Human Genetics and Anthropology, University of Jena, Jena, Germany

³Institute of Neuropathology, University of Göttingen, Göttingen, Germany

⁴Department of Trauma, Hand, and Reconstructive Surgery, University Hospitals of Ulm, Ulm, Germany

Cytogenetic information on chordomas is rudimentary and restricted to GTG-banding analysis of 26 cases worldwide. In this study, we present the chromosomal imbalances detected in a series of 16 chordomas (10 sacrococcygeal, five sphenoccipital, and one spinal) from 13 patients using comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH). On average, 3.2 losses and 4.2 gains were detected per tumor. The most common DNA copy number alterations were losses on chromosomal arms 3p (50%) and 1p (44%). Losses of 3p were detected in five of seven primary chordomas. Therefore, the loss of 3p might be an early event in chordoma genesis. The most common gains involved 7q (69%), 20 (50%), 5q (38%), and 12q (38%). Additionally, we raised the first human chordoma cell line, U-CHI, from a recurrence of a sacral chordoma. U-CHI and its parent tumor had almost the same CGH profile. According to GTG-banding and multicolor FISH, U-CHI has the following clonal chromosomal abnormalities: der(1)t(1;22), del(4), +del(5), +del(6), +7, del(9), del(10), +der(20)t(10;20), +21. Thus, the novel permanent human chordoma cell line U-CHI has chordoma-typical cytogenetic aberrations. Our data suggest that tumor suppressor genes or mismatch repair genes (located at 1p31 and 3p14) and oncogenes (located in 7q36) might be involved in chordoma genesis. © 2001 Wiley-Liss, Inc.

INTRODUCTION

Chordoma is a rare tumor that accounts for 1–4% of primary malignant bone tumors in major series (Dorfman and Czerniak, 1997). Chordomas are slow-growing, locally invasive, and potentially metastasizing neoplasms. These tumors are attributed to neoplastic transformation of notochordal remnants and occur along the axial skeleton (45% sacrococcygeal, 40% sphenoccipital, 15% spinal) (Burger et al., 1991). So far, there have been 10 studies focusing on the cytogenetics of chordomas (Persons et al., 1991; DeBoer et al., 1992; Gibas et al., 1992; Bridge et al., 1994; Mertens et al., 1994; Butler et al., 1995; Naka et al., 1996; Stepanek et al., 1998; Buonamici et al., 1999; Dalpra et al., 1999) (Table 1). Using conventional GTG-banding, these authors found different abnormal karyotypes in 13 of 26 (50%) chordomas. The majority of abnormal karyotypes were hypodiploid or nearly diploid and showed a wide variety of chromosome aberrations, but no common tumor-specific rearrangements have been noted in these studies. Overall, chordomas are considered to be heterogeneous. To date, no chordoma cell line has been

published. We present here the results of a comparative genomic hybridization (CGH)-based interphase cytogenetic study of 16 chordomas. From one of these tumors we raised the first human chordoma cell line, U-CHI.

MATERIALS AND METHODS

Tumor Samples

We studied 16 chordomas (five sphenoccipital/clivus, one spinal, 10 sacrococcygeal/sacral) from 13 patients (median age at diagnosis, 61 years; nine recurrences and seven primary tumors) (Table 2). The samples were obtained from the archives of the Institute of Neuropathology, University of Göttingen, and the Institute of Pathology, University Hospitals of Ulm. Histologic evaluation of these

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*Correspondence to: Stefanie Scheil, M.D., Institute of Pathology, University of Ulm, Albert-Einstein-Allee 11, D-89081 Ulm, Germany. E-mail: stefanie.scheil@medizin.uni-ulm.de

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TABLE I. Review of the Literature: GTG-banding Data of 13/26 Chordomas with Abnormal Karyotype*

Reference (total number of cases)	Case/sex/age	Tumor status	Local-ization	Karyotype
Persons et al., 1991 (n = 2)	1/M/56 2/F/77	R R	Sacral Sacral	44,XY,t(1;3)(q42;q11),-2,der(7)t(2;7)(q23;q32),-21/46,X,t(Y;8)(q12;q22),t(1;14)(p34;q32),t(5;10)(q13;p11)
Gibas et al., 1992 (n = 2)	1/F/71 2/F/75	P P	Sacral Sacral	36,X,-X,-1,-3,-4,-11,-13,-14,-18,-21,-22,+der(21)t(1;21)(q21;q22) 72,XX,-X,-1,+del(1)(p22)×2,-2,-3,add(3)(p25),-4,del(5)(p13),add(5)(p15),add(5)(p13),-7,inv(7)(q11.2q22),der(9)t(9;?)×2,-10,-10,-10,+12,-13,-13,der(15)t(15;?)(p11;?),-17,der(18)t(18;?)(p11;?),der(19)t(19;?)(q13;?),der(20)t(20;?)(q13;?),+der(20)t(20;?)(q13;?)-21,+der(21)t(2;21)(q11;q22)×2,+9mar[cp]
DeBoer et al., 1992 (n = 1)	1/M/69	P	Sacral	43,XY,-2,-3,del(4)(q32),-6,+7,-11,der(12)t(9;12)(q12;p11),add(16)(q23),-20,add(22)(q13),+mar
Bridge et al., 1994 (n = 1)	1/M/70	P	Sacral	42,XY,add(1)(p11),-3,der(4)t(4;?)(18;?)-6,-9,-14,der(16)t(4;?)(16)(q12;?)(q11),der(17)add(17)(p12)t(17;18)(q11;p11),der(18)del(18)(q?) [6]/42,idem,der(9)t(6;9)(q11;p11)[4]
Mertens et al., 1994 (n = 8)	6/M/51 7/M/61 8/M/62	R P R	Sacral Sacral Sacral	42,XY;add(1)(q31),del(2)(p21),-3,add(3)(p11),-4,t(5;7)(q33;q36),add(8)(q24),del(9)(p13),-10,add(11)(q11),dup(12)(q13q24),-16,-18,ins(18;t)(q21;?),add(19)(p13),der(22)t(4;22)(q11;p11),+mar[3]/46,Y,del(X)(q24),t(1;5)(p36;q33),del(2),der(3)t(3;14)(p21;q24),t(X;3)(q24;q11),der(6)t(3;6)(p21;p21),del(9),-10,add(11),del(12)(q13q15),+add(12)(q24),der(14)t(3;14)(q21;q24),der(15)t(6;15)(p21;p13),-16,?add(17)(p11),add(19),+mar[4]/48,XY,del(2),der(2)t(2;?)(12)(p14;?)(q13),inv(4)(p16q31),add(5)(p15),+7,+8,del(9),-10,del(11)p12),del(12),add(16)(p13),add(17)(q21),add(19),+mar[4]
Butler et al., 1995 (n = 5)	3/F/34	P	Sacral	46,X,-X,t(5;12)(q13;p13),t(6;7)(q25;q22),+14[1]/46,XX[19]
Buonamici et al., 1999 (n = 5)	4/M/47	P	Spinal	46,XY,t(6;11)(q12;q23)[5]
Dalpra et al., 1999 (n = 1)	A/M/39 17 months later	1 st R 2 nd R	Clivus Clivus	39,XY,dic(1;9)(p36.1;p21),add(1)(p12),del(3)(p13),-4,-4,der(6)t(1;6;14)(6pter → 6q22::1p36 → 1p13::14pter → 14qter),-7,-8,-8,-11,-17,-17,-18,-18,-19,-20,-20,-22,-22,+8mar 46,XY[18]/40,XY,del(2)(p12pter),del(3)(q21qter),del(3)(q21qter),-3,-6,-9,del(12)(q21qter),-13,-13,-15,-19,-19,-20,+mar1,+mar2,+mar3/45,X,-Y,add(6)(p25)/46,XY,del(7)(q32qter)/47,XY,+mar1/48,XY,+r1,+r2,(ace and dmin)/44,X,-Y,-2,del(2)(q31qter),t(2;Y)(p25;q11.2),add(10)(q26),-13,-14,-15,del(15)(q15ter),+20,+22,+mar1/45,XY,-4,add(10)(q26)/46,XY,del(6)(q21qter),add(D)(q?)/46,XY,add(6)(q27),add(10)(q26)/44,XY,-D,-16,-17,-18,+21,+mar1/45,XY,inv(1)(p21;q32-44),-4,-20,+mar/46,XY,add(1)(q21),add(10)(q26)/48,XY,del(3)(q21qter),-7,add(9)(q34),t(9;10)(q11;p15)+add(20)(q13.3)/43,XY,del(2)(p12pter),-6,add(10)(p15),-11,-13,-16,+mar1/46,XY,del(2)(p12pter),add(11)(q14 o q22)/46,XY,-2,-7,t(15;15)(p?;p?)-19,+mar1,+mar2,+mar3/46,XY,-4,+add(10)(q26),del(12)(q22qter)/45,Y,-X,add(1)(p12),-20,+mar/45,XY,del(6)(q25qter),del(10)(q23-24qter),-12,der(16)t(q12-13;q23-24)/46,XY,del(3)(q21qter),add(9)(q34)/44,XY,add(1)(p?),del(2)(p12pter),-6,-8,-11,-18,+mar1,+mar2/46,XY,-2,add(11)(p?)-12,+iso(12p),+mar1/43,XY,-2,add(3)(p23),-4,-21/46,XY,-6,+mar1/45,XY,del(3)(p21),-16,+mar1/43,XY,del(1)(q1qter),add(1)(p12-p13)[2],-15,-17,-20/46,XY,add(6)(q27),del(4)(q21.3qter) or t(4;6)(q21.3;q27)/44,XY,del(4)(p12),-5,t(5;11)(q12;p15),-7,-8,+10/46,XY,add(10)(q26)[2]/46,XY,+mar1/46,XY,del(2)(p12pter),add(16)(p12-p13)/47,XY,t(1;10)(p36;q24)

*M, male; F, female; P, primary chordoma; R, recurrence.

TABLE 2. Summary of Selected Clinical Data, Histopathologic Characteristics, and Results of CGH and FISH Analysis of 16 Chordomas from 13 Patients*

Case/sex/age/ tumor status	Localization	Ki-67 PI	FISH		Chromosomal imbalances using CGH	
			(Mean of FISH signals/nucleus)		Gains	Losses
1/M/46/R	Sacral	13.4	1p22: 1.9; 1p36: 1.8; 3p14: 1.9; 6cen: 2.7; 7cen: 4.7; 7q34-q35: 4.2; 7qTIM: 4.6; 7q36: 4.7; 7cen: NA; 7q34-q35: 2.5; 1p36: 3.1; 6cen: 1.6; 7cen: 2.8; 1p36: 3.4; 7cen: 3.2; 7q34-q35: 3.9; 1p22: 2.1; 7cen: NA; 7qTIM: 2.5; ND	7qter: 4.7; 8p12: 3.8; 9cen: 2.7; 9q34: 3.6; 10cen: 1.4; 22q11: 2.0; 22q12: 1.9	7; 8p9q34 ; 12q34; 15q; 17, 20 q	1p34-p21 ; 3 ; 10 ; 11; 14q; 18; 22
2/F/77/R	Sacral	5.5	7q34-q35: 2.5; 1p36: 3.1; 6cen: 1.6; 7cen: 2.8; 1p36: 3.4; 7cen: 3.2; 7q34-q35: 3.9; 1p22: 2.1; 7cen: NA; 7qTIM: 2.5; ND	7qter: 2.7	7q36 , 20	1p31.3-p22; 3p21-p12; 13q21-q32; 18q22-q23
3/F/70/P	Sacral	14.4	7q34-q35: 2.5; 1p36: 3.1; 6cen: 1.6; 7cen: 2.8; 1p36: 3.4; 7cen: 3.2; 7q34-q35: 3.9; 1p22: 2.1; 7cen: NA; 7qTIM: 2.5; ND	7qter: 2.9; 9q34: 1.9; 22q11: 2.8	1p34.2-p36 ; 7p21-qter ; 12p; 15q; 22q	1p31-p21; 3p; 6q11-q21 ; 9p; Xp
3R/F/71/R	Sacral	14.2	7q34-q35: 2.5; 1p36: 3.1; 6cen: 1.6; 7cen: 2.8; 1p36: 3.4; 7cen: 3.2; 7q34-q35: 3.9; 1p22: 2.1; 7cen: NA; 7qTIM: 2.5; ND	7qTIM: 3.8; 7q36: 3.9	amp1p34.2-p36 ; 7 ; 12p; 15q; 22q	1p31-p21; 2q33-q36; 3p; 6q11-q21; 9p-q31; Xp
4/F/69/P	Sacral	ND	7q34-q35: 2.5; 1p36: 3.1; 6cen: 1.6; 7cen: 2.8; 1p36: 3.4; 7cen: 3.2; 7q34-q35: 3.9; 1p22: 2.1; 7cen: NA; 7qTIM: 2.5; ND	7q34-q35: 3.0; 7q36: 3.3	7q22-qter ; 12p	
4R/F/70/R	Sacral	4.5	6cen: 2.8; 9q34: 2.7; 7cen: NA; 7q34-q35: 2.5; 7cen: NA; 7q36: 2.4; 1p22: 2.2; 3p14: 4.2; 7cen: NA; 7q34-q35: 4; 7qTIM: 4.5; 9q34: 2.6; ND	10cen: 2.8; 22q11: 2.6 7q36: 2.9	7q22-qter 17; 20q	3; 4; 5; 9p; 10 1p31; 4p; 9p21-p24; 13q21
5/F/61/R	Sacral	5.5	7q34-q35: 2.5; 7cen: NA; 7q36: 2.4; 1p22: 2.2; 3p14: 4.2; 7cen: NA; 7q34-q35: 4; 7qTIM: 4.5; 9q34: 2.6; ND	7q36: 2.9	5q23-qter; 7 ; 12q24; 20	3; 4q35
6R/F/52/R	Sacral	2.1	7q34-q35: 2.5; 7cen: NA; 7q36: 2.4; 1p22: 2.2; 3p14: 4.2; 7cen: NA; 7q34-q35: 4; 7qTIM: 4.5; 9q34: 2.6; ND	9q34: 2.2; 22q11: 2.7	5q31-qter; 7q34-qter ; 12q24; 20; 22q ; Xq23-qter	—
7/F/74/R	Sacral	4.5	7q34-q35: 2.5; 7cen: NA; 7q36: 2.4; 1p22: 2.2; 3p14: 4.2; 7cen: NA; 7q34-q35: 4; 7qTIM: 4.5; 9q34: 2.6; ND	7q36: 4.1; 8p12: 4.1; 10cen: 2.3; 22q12: 4.3	1q; 3p , 4q12-q27, 5q; 7 , 8ppter-q21.1 , 8q24, 9q22-qter, 11pter-q22, 12, 13q22-qter, 15q, 17q, 21, 22	—
8/M/26/P	Clivus	1.4	6cen: 2.5; 7cen: 2.6; 7q34-q35: 2.9; 1p22: 2.4; 7cen: NA; 7q34-q35: 2.3; 9q34: 2.7; 7cen: NA; 7q34-q35: 2.8; 7q36: 2.8;	22q11: 3.5	20; 21q22; 22q ; Xp, Xq26-q28 11q24-q25; 12q24; 14q21-qter; 17q; 20q; 21q21-q22 7q34-qter ; 20q	— 6q; 12p; 13q 1p31-p21; X
9/F/66/R	Clivus	ND	6cen: 2.5; 7cen: 2.6; 7q34-q35: 2.9; 1p22: 2.4; 7cen: NA; 7q34-q35: 2.3; 9q34: 2.7; 7cen: NA; 7q34-q35: 2.8; 7q36: 2.8;	7q36: 2.9; 10cen: 2.5	12q24	13q21-q31; Xq25-Xqter
10/F/51/R	Clivus	4.4	6cen: 2.5; 7cen: 2.6; 7q34-q35: 2.9; 1p22: 2.4; 7cen: NA; 7q34-q35: 2.3; 9q34: 2.7; 7cen: NA; 7q34-q35: 2.8; 7q36: 2.8;	7q36: 2.4; 9q34: 1.9; 22q11: 2.1 22q11: 2.8 9q34: 2.9; 22q11: 3.2		
11/M/37/P	Clivus	5.7	6cen: 2.5; 7cen: 2.6; 7q34-q35: 2.9; 1p22: 2.4; 7cen: NA; 7q34-q35: 2.3; 9q34: 2.7; 7cen: NA; 7q34-q35: 2.8; 7q36: 2.8;	7q36: 2.4; 9q34: 1.9; 22q11: 2.1 22q11: 2.8 9q34: 2.9; 22q11: 3.2		
12/F/58/P	Clivus	3.2	6cen: 2.5; 7cen: 2.6; 7q34-q35: 2.9; 1p22: 2.4; 7cen: NA; 7q34-q35: 2.3; 9q34: 2.7; 7cen: NA; 7q34-q35: 2.8; 7q36: 2.8;	1q; 11q24-q25 5p15; 7q34-qter ; 9q34 ; 22q		1p; 3; 4; 9p; 10; 13q; 14q; X 3p12-p14; 13q; 18q
13/F/80/P	Spinal	2	6cen: 2.5; 7cen: 2.6; 7q34-q35: 2.9; 1p22: 2.4; 7cen: NA; 7q34-q35: 2.3; 9q34: 2.7; 7cen: NA; 7q34-q35: 2.8; 7q36: 2.8;	22q11: 3.2		

*M, male; F, female; P, primary chordoma; R, recurrence and radiotherapy before surgery; FISH, fluorescence in situ hybridization; PI, proliferation index; NA, not available; ND, no data. CGH data confirmed by FISH are given in boldface type.

samples showed an estimated tumor cell content of 80% of the tissue volume. Paraffin-embedded material was prepared as follows: 10 serial 4- μ m-thick sections for immunohistochemistry, followed by one 40- μ m cut for nucleus extraction and fluorescence in situ hybridization (FISH) and 10 serial 10- μ m-thick sections for DNA extraction and CGH. A final section was stained with hematoxylin and eosin.

Immunohistochemistry and Ki-67 Proliferation Index

Immunostaining was done using an indirect peroxidase method. The following antibodies were used: rabbit polyclonal antibody against S-100 protein (Dako, Copenhagen, Denmark) and mouse monoclonal antibodies against pan-cytokeratin (MNF116, Dako), epithelial membrane antigen (EMA; E29, Dako), and vimentin (3b4, Linaris, Wertheim, Germany). All monoclonal antibodies were used at a final concentration of 1–2 μ g/mL. All tumors were evaluated on formalin-fixed paraffin sections for the expression of the proliferation-associated nuclear antigen Ki-67 (clone MIB-1; Dianova, Hamburg, Germany). The Ki-67 proliferation index was evaluated by counting labeled nuclei in five different high-power fields in the tumor area with the highest density of labeled cells. The labeled nuclei counted were expressed as a percentage of tumor nuclei.

Cell Culture

The human chordoma cell line U-CH1 (Fig. 1A) was established from a recurrence of a sacrococcygeal chordoma (case 1; Table 2; Fig. 1B). The tumor was a local recurrence after radiotherapy of a chordoma 4 years after initial surgery. Material from the primary tumor was not available. The 46-year-old male patient gave his informed consent to the establishment of a permanent tumor cell line. The primary cells were seeded in collagen-coated flasks in Iscove/RPMI (4:1) medium (Boehringer, Ingelheim, Germany) containing 10% fetal calf serum, 2 mM glutamine, and antibiotics (100 U/mL penicillin G, 100 μ g/mL streptomycin). After confluence, these cells were subcultivated by trypsin/EDTA (0.25%/0.02%, Boehringer). At present, U-CH1 has been growing for more than 1 year in culture and has a generation time of about 1 week.

Chromosome Preparation

Metaphase chromosome spreads were prepared from U-CH1 cells or from primary blood cell cul-

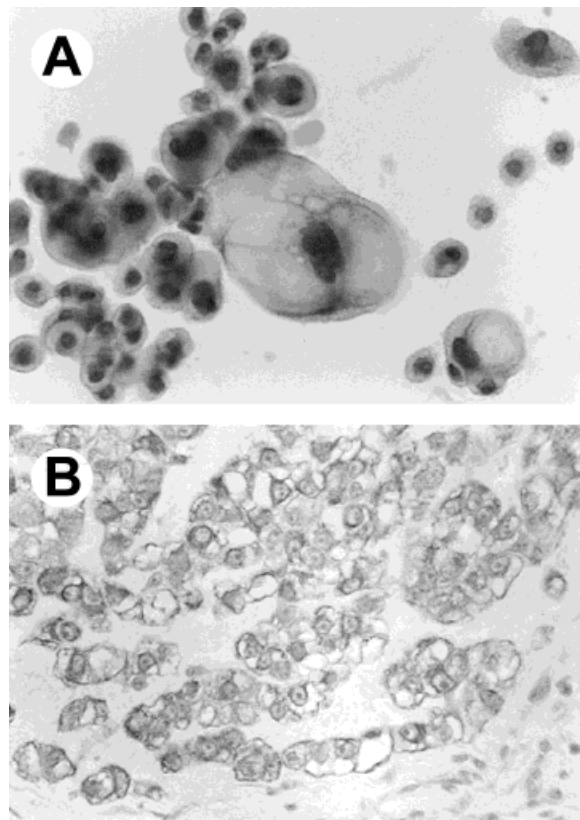


Figure 1. Morphologic features of U-CH1 and its parental tumor. **A:** Cytologic characteristics of U-CH1 cell line grown on chamber slides. Physaliferous cells of different sizes with round nuclei and cytoplasmic vacuoles (hematoxylin and eosin $\times 290$). **B:** Immunohistochemical staining of the parental tumor of U-CH1 (case 1) shows strong positivity for pan-cytokeratin and typical chordoma features, mainly consisting of physaliferous cells surrounded by a mucinous intercellular substance ($\times 290$).

tures of healthy donors (for CGH experiments) using standard protocols (Scheil et al., 2001). Tumor cells were karyotyped by conventional GTG-banding techniques according to the 1995 International System for Human Cytogenetic Nomenclature.

DNA Preparation and CGH

All tumor samples were available as formalin-fixed and paraffin-embedded material. In part, the DNA was extracted from fresh-frozen tissue (case nos. 1-4R, 6R, 7, 9, 12). For DNA extraction, the commercially available DNA extraction Clean-Mix kit (Talent, Trieste, Italy) was used. The control DNA was isolated from fresh-frozen material of normal human tonsils by phenol/chloroform extraction. Nick translation of DNA probes and CGH analysis were carried out according to the protocol previously described in detail (Scheil et al., 2001). If only small amounts of DNA were available, de-

generate oligonucleotide primed polymerase chain reaction techniques were used, in order to amplify and label DNA for CGH analysis (Telenius et al., 1992). Image acquisition and processing were performed with the image analysis system ISIS (MetaSystems, Altussheim, Germany). Mean ratio profiles were detected from the analysis of at least 12 metaphase spreads. CGH ratio values of 1.25 and 0.8 were used as the upper and lower thresholds, respectively, for the identification of chromosomal imbalances. CGH experiments were carried out along with control experiments in which differently labeled normal DNA was hybridized to normal metaphase chromosomes. The control experiments showed no chromosomal imbalances.

Fluorescence In Situ Hybridization

FISH was done in 14 of 16 cases (Table 2). In 13 of 14 cases, the material was paraffin-embedded, and this material was limited. Therefore, we had to select a small number of FISH loci. Selection of loci was done according to the results of CGH. Nuclei from paraffin-embedded tumors were isolated from 40- μ m sections according to the protocols of Liehr et al. (1995). Cytospin slides were made from isolated nuclei, and the slides were stored at -20°C . For FISH analysis of interphase nuclei extracted from paraffin-embedded tissue, we modified digestion times (pepsin, 10–20 min; proteinase K, 60–120 min), using the same enzyme concentrations as previously mentioned (Liehr et al., 1995). FISH analysis of interphase nuclei or metaphase spreads of U-CH1 was performed as previously described (Scheil et al., 2001).

Digoxigenin-labeled probes were visualized using a Cy3-conjugated monoclonal mouse anti-digoxigenin antibody (IgG; Jackson Immuno-Research Laboratories Inc., West Grove, PA). Biotin-labeled probes were visualized using fluorescein isothiocyanate-conjugated avidin (Vector, Burlingame, CA). At least 50 nuclei (in most cases 100 nuclei per probe) were evaluated. We were particularly interested in probes assigned to chromosomal arms 1p, 7q, and 22q. The following commercially available digoxigenin- or biotin-labeled probes were used with assignment to human chromosomes: 1p36, 6cen, 7cen, 9cen, 10cen, and the combined probe m-bcr/abl, with assignment to 9q34 (*ABL* locus) and 22q11 (*BCR* locus) (all probes by Q-Biogene, Illkirch Cedex, France). Additionally, we used the directly labeled TelVysion 7q probe (Vysis, Downers Grove, IL). We also selected representative clones from a panel of YAC clones (Huang et al., 1996; Doehner et al., 1998;

Ross et al., 1998). We used the following YAC clones obtained from the CEPH YAC library: 848_E_3 (1p22), 801_A_8 (3p14), 940_A_12 (7q34-q35; *NEDD2*, *TRCB*, *PRSSI*, *KEL*, *PIPI*, *CLCN1*), 761_H_5 (7q34-q35, *TIMI*), 724_G_5 (7q36, *RHEB*), 798_G_8 (8p12), 763_A_3 (22q12), 744_F_6 (22q12). Detailed information on the YACs is available at <http://www.cephb.fr/cephgenethon-map.html>. The YAC clones were prepared as described elsewhere (Doehner et al., 1998).

FISH experiments were performed as dual-color hybridization. The cutoff level was defined by the mean + 3 SE of the frequency of control cells exhibiting only one fluorescence signal or more than two. The cutoff levels were determined for five representative, commercially available probes or YACs from the YAC map (range, 2.9–13.8% for losses and 9.6–23.9% for gains). Signal numbers were enumerated in 100–200 nuclei. Multicolor FISH (M-FISH) was performed as described by Senger et al. (1998). Labeled metaphase cells were analyzed in a Zeiss Axioskop microscope (Jena, Germany) equipped with a CCD camera and linked to ISIS version 3 software (MetaSystems).

Statistics

All statistical tests were two-sided. CGH data of the subgroups (sphenoccipital versus sacrococcygeal and primary versus recurrence) were tested using Wilcoxon's rank sum test. We calculated the mean, standard error of the mean (SE), and median using Microsoft Word Excel software (version Excel 97).

RESULTS

CGH Findings on Sixteen Chordomas

The CGH findings on 16 chordomas are shown in Table 2 and Fig. 2. Overall, we found 117 chromosomal aberrations (two to 14 per tumor; median, six per tumor). On average, 3.2 losses and 4.2 gains were detected per tumor.

Statistical analysis showed no significant difference in the number of chromosomal imbalances detected by CGH between chordomas of different localization—sacrococcygeal (median, six per tumor) compared with sphenoccipital (median, four per tumor)—or between primary chordomas (median, six per tumor) and recurrences (median, six per tumor). The proliferation index of the tumor and the number of imbalanced chromosomes per tumor were not correlated. Losses of DNA sequences were most prevalent at 3p (8/16) and 1p

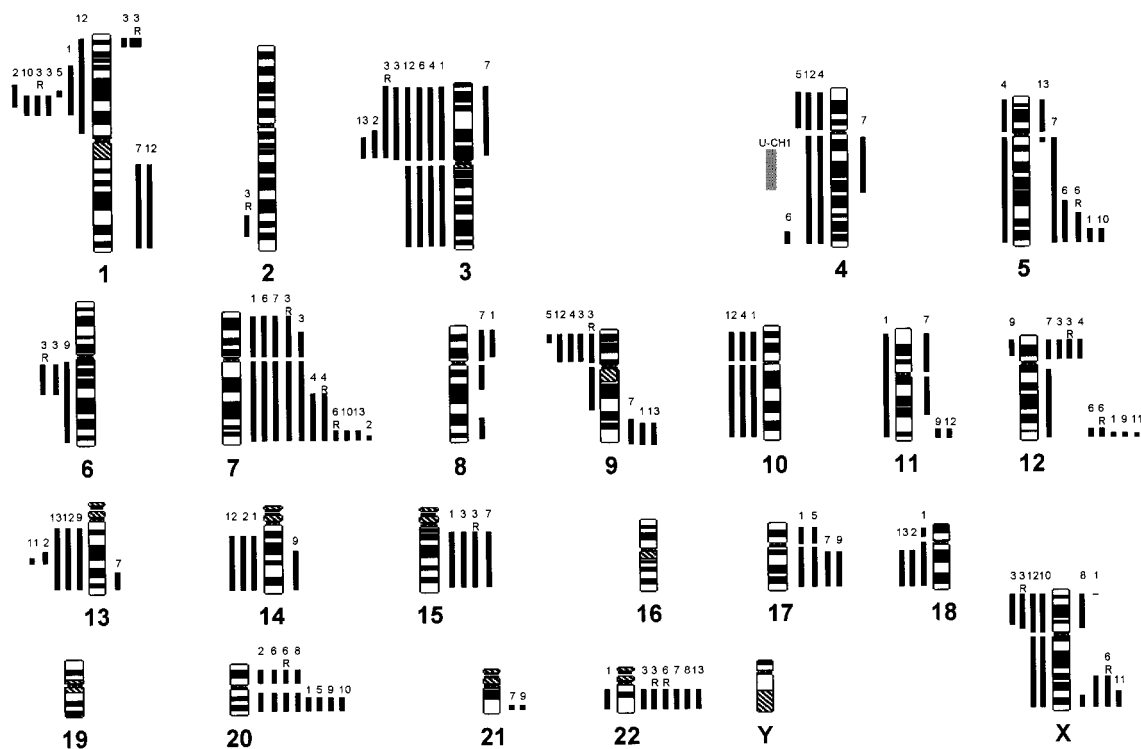


Figure 2. Summary of all DNA sequence copy number changes detected by CGH in 16 cases (13 patients) of chordoma. Vertical lines on the left side of the chromosome indicate losses, and vertical lines on the right correspond to gains. Chromosomes 16 and 19 were not evaluated. The numbers above each line refer to the case analyzed. The bold line at 1p34.3-p36 represents high-level DNA amplification. The only difference between U-CH1 and its parental tumor (case 1) was a loss of 4q13-q28 in the cell line (vertical gray line on the left side of chromosome 4).

(7/16). Losses of 3p were detected in five of seven primary chordomas. Loss of 13q was found in one of 10 sacrococcygeal tumors, in the single spinal tumor, and in three of five sphenococcygeal tumors. DNA sequence copy number gains occurred most frequently at 7q (11/16), 20 (8/16), 5q (6/16), and 12q (6/16). Gains of chromosome 17 and the majority of gains of 5q, 12q, and 20 were found in recurrences. The distribution of deletions and gains and the total number of aberrations in tumors are shown in Table 2 and Figure 2.

FISH Analysis of Archival Chordomas

FISH was performed on 14 of 16 samples. The loci were selected according to the results of CGH. The FISH experiments did not show further chromosomal alterations and confirmed the CGH data (Table 2). In four of 11 cases, the gain of chromosomal material on 7q was terminally accentuated. Terminal gains detected by CGH are difficult to interpret. Hence, in 11 of 14 samples, FISH probes for 7q were used, confirming the CGH results (Table 2). One high-level gene amplification at 1p34.3-

p36 was detected in a local recurrence of a sacral chordoma (case 3R). FISH analysis of 1p36 showed more than three signals per nucleus in 49% of the cells (10 or more in 21% of the nuclei). The high-level amplification of case 3R was not detectable in the primary lesion (case 3), obtained before radiation therapy.

Morphologic Characteristics of the Chordoma Cell Line (U-CH1)

U-CH1 cells were physaliferous with variably abundant cytoplasm, cytoplasmic vacuoles, and round nuclei, thus featuring the typical cytomorphic features of chordomas (Fig. 1A). U-CH1 had an immunocytochemical profile usually found in chordomas: co-expression of S-100 protein, vimentin, EMA, and cytokeratin (data not shown). On histology, the parental tumor of U-CH1 (case 1) showed typical chordoma features, mainly consisting of physaliferous cells with mucinous intercellular substance. The immunohistochemical profile was identical to that of U-CH1 (Fig. 1B).

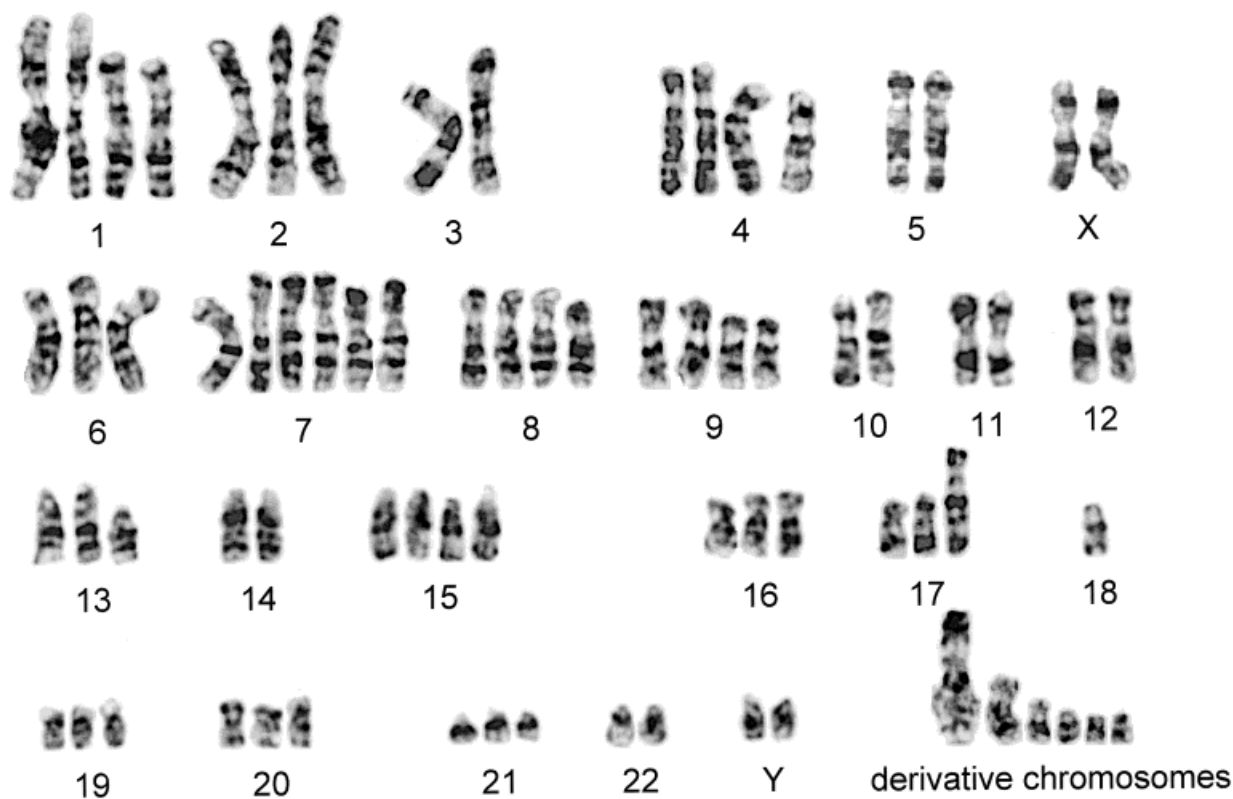


Figure 3. One representative karyotype of U-CHI: 75,XXY,+Y,+1,t(1;22)(p11;q?) \times 2,-3,+del(4)(q13q28),-5,+7,+7,+7,+8+9,del(9)(p12p22) \times 2,-10,t(10;20)(p11.2;q?) \times 2,del(13)(q12q13),-14,+15,t(17;17)(q11;q23),-18,-18,-22,+6mar.

Interphase Cytogenetics of U-CHI and Its Parental Tumor

We compared the DNA extracted from U-CHI at two different times (cell cycle passages 8 and 20, respectively) with the DNA of the parental tumor (case 1). Using CGH analysis, we found nearly identical profiles, and they showed almost the same *rev ish* karyotypes, *enh*(7,8p,9q34,12q24,15,20q), *dim*(1p21-p34,3,10,11,14,18,22). The only difference between U-CHI and its parental tumor was a loss of 4q13-q28 in the cell line (Table 2). Interphase FISH analysis of U-CHI was performed at passage 5. On average, losses seen at CGH analysis had 1.4–2.0 signals/nucleus and gains had 3.6–4.8 signals/nucleus (Table 2). The only discrepancy between FISH and CGH was that FISH showed the same number of signals for the 1p22 probe and the 1p36 probe (Table 2).

Karyotype of U-CHI by Chromosome Banding Analysis and M-FISH

Twenty-three G-banded metaphase cells were analyzed (cell cycle passage 4). No cell was karyotypically normal. One cell population was hypodiploid

(40–43 chromosomes), and one part was hypertriploid (72–79 chromosomes). All GTG-banded cells had the following clonal abnormalities: del(1)(p), del(4), +7, add(10)(p13), +21 (Fig. 3). M-FISH was done on four metaphases (cell cycle passages 10–12) (Fig. 4). Based on GTG-banding and M-FISH, the following karyotype emerged: 40–43,XY,der(1)t(1;22),del(4),+del(5),+del(6),+7,del(9),del(10),+der(20)t(10;20),+21/72–79,XXY,+Y,+1,der(1)t(1;22) \times 2,-3,+del(4),+5,del(5) \times 2,+6,del(6) \times 2,+7,+7,+7,?del(8),+8 or +?del(8),del(9),+10,del(10) \times 2,-11,-12,-14,+15,-17,-18,-19,der(20)t(10;20),+21,-22.

DISCUSSION

Of the 26 cases of cytogenetically analyzed chordomas reported in the literature, 13 tumors had clonal abnormalities (Persons et al., 1991; DeBoer et al., 1992; Gibas et al., 1992; Bridge et al., 1994; Mertens et al., 1994; Butler et al., 1995; Naka et al., 1996; Stepanek et al., 1998; Buonamici et al., 1999; Dalpra et al., 1999) (Table 1). In contrast, in our cohort of 16 chordomas from 13 patients, we found imbalances in every tumor. Gains were more com-

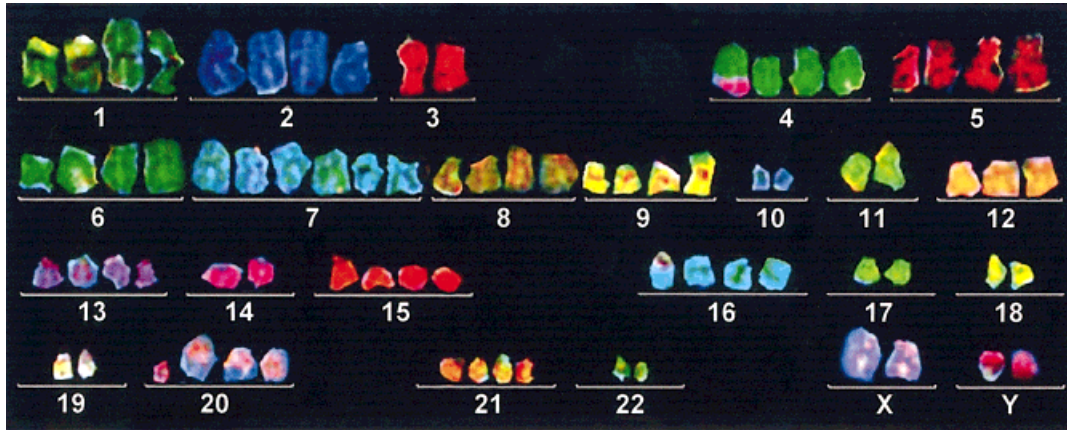


Figure 4. One M-FISH karyotype of a hyperdiploid metaphase spread of U-CH1: 77,XXY, +Y, +1,der(1)t(1;22)×2, +2, -3,del(4), +t(4;14), +del(5), +6,del(6)×2, +7, +7, +7, ?del(8), +8 or +?del(8), +9, del(9)×2, -10, del(10)×2, -11, ?del(11), +13, -14, +15, +16, t(16;19), -17, -18, -19, der(20)t(10;20)×2, +?del(20), +21, -22.



Figure 5. Summary of the most frequently found chromosomal imbalances. Bold lines indicate our own data by interphase cytogenetics of 16 chordomas and U-CH1; dashed lines and asterisks indicate cytogenetic data from published cases of chordoma. Lines on the left side of the chromosome indicate losses, and lines on the right side correspond to gains. The bold line on the right of 1p34.3-p36 represents high-level DNA amplification.

mon (1.3:1) than losses at CGH. There was no significant difference between subgroups (sphenoccipital vs. sacrococcygeal; primary vs. recurrence). In our cohort, recurrent chordomas, which were obtained, as a rule, after radiotherapy, did not have more chromosomal imbalances than primary chordomas.

Most abnormal karyotypes reported in the literature were hypo- or near diploid, but two had near triploid clones (Table 1). On the basis of FISH analysis combined with CGH data, we estimated

that approximately one-third of the cases in our series were hyperdiploid (two primary lesions and three recurrences). Thus, it may be that hypo- or near diploid cell populations have a growth advantage in vitro.

Using GTG banding and M-FISH, U-CH1 showed the following clonal chromosomal abnormalities: der(1)t(1;22), del(4), +del(5), +del(6), +7, del(9), del(10), +der(20)t(10;20), +21. According to the literature, structural chromosomal aberrations were found in nearly all chordomas with

aberrant karyotypes (Table 1), with five abnormalities involving 1p36. One loss-of-heterozygosity study by Miozzo et al. (2000) found evidence of a tumor suppressor gene mapping to 1p36, involved in familial and sporadic chordomas. Although the U-CH1 cell line had no breakpoint in 1p36, CGH, FISH, and M-FISH all indicated a shortened p arm (Fig. 4). Using CGH, underrepresentation of chromosomal material was most frequently found at 1p and 3p. These data match published cytogenetic observations showing loss of 3p in five of seven primary chordomas. Thus, loss of 3p might be an early event in chordoma genesis.

Steilen-Gimbel et al. (1999) showed that an unbalanced translocation t(1;3) is associated with chordoid differentiation in meningiomas. It remains to be elucidated whether a fusion gene and/or combined loss of 1p and 3p material are the biological determinants of this phenotype. Chordoid gliomas do not show any chromosomal imbalance using CGH (Reifenberger et al., 1999). We found combined losses of 1p and 3p in four cases. In our study, the most frequently detected gain of chromosomal material was found on chromosome arm 7q. The consensus region for gains on the long arm of chromosome 7 was 7q36. Candidate genes on 7q36 include the homeobox-containing gene *HLXB9* and sonic hedgehog gene (*SHH*). Both are expressed throughout the notochord and its caudal end during embryogenesis (Ross et al., 1998).

In conclusion, our study showed that every chordoma had chromosomal imbalances (median, six per tumor). There were no significant differences between primary chordomas and recurrences or among tumors of different anatomic locations. We extended and confirmed published data that 1p and 3p are chromosomal regions frequently involved in imbalances (Fig. 5). In our cohort, 1p and 3p were most often affected by losses, whereas chromosomal gains were most common on 7q. Like its parental tumor, U-CH1, the first permanent human chordoma cell line, is cytogenetically highly characteristic and is thus a suitable tool for further elucidation of the molecular cytogenetics and oncogenesis of chordoma.

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