Identification of isochromosome 1q as a recurring chromosome aberration in skull base chordomas: a new marker for aggressive tumors?

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Object. The authors conducted a study of 22 skull base chordomas.

Methods. A series of 22 skull base chordomas was analyzed with G banding. Subsequently, metaphase cells obtained from three tumors were reexamined using multicolor spectral karyotyping. Clonal chromosome aberrations were identified in 11 cases, all of which were recurrent tumors. Three tumors showed a remarkable similarity in cytogenetic features, and these features appear to characterize a recurring combination of nonrandom chromosome aberrations, including isochromosome 1q, gain of chromosome 7, and monosomy for chromosomes 3, 4, 10, 13, and 18. Isochromosome 1q was identified as the sole recurring structural chromosome rearrangement in these tumors. The pattern of chromosome loss reported in the progression of lumbosacral chordoma also appears to be true of skull base chordomas with the additional findings of isochromosome 1q, gain of chromosome 7, and loss of chromosome 18.

Conclusions. Skull base chordomas characterized by isochromosome 1q and monosomy 13 provide support for the concept of the loss of putative tumor suppressor loci on 1p and 13q and aggressive tumor behavior.

KEY WORDS • chordoma • chromosome aberration • isochromosome 1q • spectral karyotyping • cytogenetics

Chordomas are locally invasive bone tumors that appear in adulthood and are thought to originate from the remnants of the notochord. Because chordomas have a predilection for the ends of the spinal column, most lesions are found in the sacrococcygeal region and the base of the skull.^{7,16,17} Approximately 35% of chordomas arise at the base of the skull, approximately 50% arise in the sacrococcygeal region, and the remaining 15% are found in the vertebral bodies.¹⁴ Skull based chordomas are slow-growing tumors and, in many cases, are associated with a significant amount of bone and soft-tissue destruction. Metastasis can occur but usually in the late stages of tumor progression. Because of their location, cranial chordomas are difficult to manage and gross-total resection is not always possible; therefore the recurrence rate is high.¹⁴ Unfortunately, the prognosis for patients with intracranial tumors is poor, as the progression of the tumor causes death in most patients.¹²

Cytogenetic studies of chordomas are limited. The great majority of cases reported are lumbosacral chordomas, which have shown diverse chromosome aberrations.^{2,3,6,8,}

^{9,13,18,21} In most cases either normal karyotypes or hypodiploidy with complex structural aberrations have been shown. The hypodiploidy has most often included loss of chromosomes 3, 4, 10, and 13.¹⁸ To date, no consistent structural chromosome aberrations have been reported.

In the present study, a series of 22 skull base chordomas was analyzed by classic G banding. In addition, metaphase cells obtained from three tumors were analyzed with SKY, which is a molecular cytogenetic technique that allows the simultaneous display of each chromosome in a different color.²⁷ This technique makes possible the identification of chromosomal bands of unknown origin, including translocations, insertions, complex rearrangements, and small marker chromosomes. We report the largest cytogenetic study of skull base chordomas and, to our knowledge, the identification of isochromosome 1q as the first recurring structural chromosome aberration in these tumors.

MATERIALS AND METHODS

Patient Population

There were 11 patients (four females and seven males) who ranged in age from 15 to 69 years. Table 1 provides a summary of clinical and histological data.

Abbreviations used in this paper: DAIP = 4,6-diamidino-2-phenylidole; LOH = loss of heterozygosity; RB = retinoblastoma; SKY = spectral karyotyping.

TABLE 1
Clinical and histological findings obtained in 11 patients with chordomas

Case No.	Age (yrs), Sex	Tumor Characteristics			No. of Ops (yr)	Radiotherapy
		Location	Recurrent	Histology		
1	57, F	clival	yes	chondroid	1 (1992)	yes
2	25, M	clival	yes	chondroid	1 (1991)	no
3	34, M	clival	yes	conventional	1 (1992)	no
4	58, F	clival	yes	conventional	1 (1994)	yes
5	41, M	clival	yes	conventional	1 (1989)	yes
6	33, M	cervical	yes	conventional	2 (1993 & 1995)	yes
7	65, M	cervical	yes	conventional	1 (1987)	yes
8	15, F	clival	yes	conventional	2 (1998 & 1998)	yes
9	52, M	clival	yes	conventional	2 (1997 & 1999)	yes
10	69, M	clival	yes	conventional	1 (1996)	yes
11	47, F	clival	yes	conventional	1 (1998)	yes

Cytogenetic Analysis

Cytogenetic analysis was performed by standard in situ culture techniques and G banding, as described elsewhere.²⁵ Chromosome aberrations have been described by the Cancer Cytogenetic Guidelines and Nomenclature of the International System for Human Cytogenetic Nomenclature.²⁰ In cases in which routine analysis of 20 colonies obtained from at least three independent primary culture slides indicated chromosome instability, additional metaphase spreads were analyzed.

Spectral Karyotyping Methods

We used prepared SKY probe mixture and hybridization reagents (Applied Spectral Imaging, Carlsbad, CA). Slides for SKY were treated according to the manufacturer's protocol: the probe cocktail was hybridized to the slides for 2 days at 37°C. Image acquisition was performed using a Spectracube (SD200; Applied Spectral Imaging, Inc.) mounted on a Zeiss Axiomat II microscope fitted with a custom-designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT) that allows for simultaneous excitation of all dyes and measurement of their emission spectra. The DAPI banding images were acquired as part of the image-acquisition process and were analyzed using a DAPI-specific optical filter. The DAPI images were used in conjunction with spectral classifications and G banding for the identification of chromosome aberrations, as previously described.²⁶

RESULTS

Twenty-four tumors were cultured and harvested. Two specimens could not be evaluated due to the absence of growth or low mitotic index. Eleven tumors showed no clonal chromosome aberrations, nine were new primary tumors, and two were recurrent. Clonal chromosome aberrations were found in 11 cases, all of which were recurrent tumors (Table 2). Five of these tumors showed multiple clones (Table 2).

Results of G Banding

Clonal chromosome losses were identified in six tumors (obtained from Cases 1–3, 5, 6, and 11) (Table 2). In four of these, losses included the deletion or loss of chromo-

somes 3, 4, 13, and 18 (Cases 1, 5, 6, and 11), followed by the loss of chromosome 10 (Cases 5, 6, and 11) in three of the four tumors (Table 2). The most common chromosome gains included all or part of chromosome 1q (in Cases 1, 5, 6, and 11) and chromosome 7 (Cases 2 and 5–7). Three tumors shared chromosome aberrations of isochromosome 1q, -3,-4,+7,-10,del13/-13, -18 (Cases 5, 6, and 11) (Fig. 1).

Results of SKY

Spectral karyotyping was used in three cases, resulting in either confirmation or refinement of aberrations previously identified by G banding. In the tumor obtained in the patient in Case 6 SKY refined the designation of an add(6)(q23) to an unbalanced translocation der(6)t(6;13)(q23;q21). This finding identified a deleted region in chromosome 13 including bands 13pter~13q14, indicating the loss of the RB region (Fig. 2). In the tumor obtained in the patient in Case 9 SKY refined the designation of an add(9)(q34) to a der(9)t(9;11)(q34;q23), and an add(19q) and del(20q) to a t(19;20)(q13;q11.2). The technique also confirmed a questionable t(8;18)(q21.2;q12) in this case. In the tumor obtained in the patient in Case 10 SKY confirmed the complex translocations demonstrated by G banding including t(3;5)(p23;p11),t(3;10)(q21;q24),t(4;6)(q31.1;p11.2), and t(4;16)(q25;p11.2).

DISCUSSION

Cytogenetic studies of skull base chordomas are rare. To our knowledge, only two recent studies have been reported.^{3,8} Four tumors were analyzed by Buonamici, et al.,³ three of which showed a normal karyotype, whereas one tumor showed a t(6;11)(q12;q23) as the sole aberration. Dalpra, et al.,⁸ reported a clonal dicentric (1;9)(p36.1;p21) cell line and involvement of 1p in unbalanced translocations that led to variable loss of 1p. This finding has more recently led to the localization of a putative tumor suppressor locus in familial and sporadic chordomas being mapped to 1p36.¹⁹ In addition to this locus, the RB tumor suppressor gene (13q14) has also been implicated in the pathogenesis of chordomas. In a screening for LOH for the RB gene, Eisenberg, et al.,¹⁰ identified two of seven chordomas in which LOH for the RB gene was demon-

TABLE 2
Cytogenetic findings in tumors with abnormal karyotypes

Case No.	Findings
1	46,XX,inv(1)(q23q42),t(1;10)(q32;p11),t(3;14)(p21;q13),inv(4)(p14q31),add(12)(q22),del(14)(q32)[5] 46~48,XX,add(1)(q?32),del(3)(p25),del(5)(q31),del(6)(q15),add(11)(p13),+del(12)(q22),-13,add(16)(p11),add(16)(q24),+17,der(18)t(1;18)(q12;q23)x2,add(19)(q13)[cp3] 46,X,del(X)(p22.1),t(1;9)(p36.1;p13),t(4;9)(p12;q34),t(6;16)(p11;q24)[2] 45,X,del(X)(p11.2p11.4),der(5)t(5;14)(p13;q11),?add(11)(q22),del(12)(q22),-13,der(17)t(13;17)(q14;p13),add(21)(q22)[3]
2	48,XY,+5,+7,+12,-13,add(13)(q34),-18,+20[2] 49,idem,+19[16]
3	44~45,XY,t(2;14)(p23;q11),?t(3;12)(p21;p13),del(4)(q?23),-5,-6,der(11)t(6;11)(q11;p12)[cp2]
4	46,XX,t(4;17)(q23;q21),t(8;9)(q11;q11)[3] 46,XX,t(2;20)(p31;p11.2),t(3;22;16)(p21;q11.2;q22),del(6)(p23)[2]
5	52~66,-X,-X,-Y,add(1)(q22)x2,i(1)(q10),+2,-3,der(3)t(3;4)(p?24;q?13)t(1;3)(q21;q21)X2,-4,der(4)del(4)(p12)add(4)(q13)x2,add(6)(q?21)+7,-9,-10,add(11)(q13)x2,add(11)(q25),+add(11)(q25),-12,-13,add(13)(?q13),?del(15)(q11.1q13)x2,?dup(15)(q11.1q13),add(17)(q24),-18,+19,-20,+21,+22[cp9] 46,XY,-1,der(1)?t(1;4)(p13;q?27),+der(3)t(3;11)(q29;q11)add(3)(p13),del(4)(p12),der(4)add(4)(p16)t(1;4),inv(7)(p?21q?34),add(8)(p22),del(9)(q21),del(10)(q22),der(11)add(11)(p15)?del(11)(q13q22),?inv(14)(q11.2q32),+add(15)(q22),der(15;16)(q10;q10),?t(17;20)(q?23;q?13.3)[cp4]
6	38,X,-X,i(1)(q10),-3,-4,add(6)(q27),+7,-9,-10,-13,-14,-18,-22[cp5]
7	43~45,-Y,del(X)(q?26),t(2;17)(q21;p13),del(6)(q?25),add(9)(p24),inv(14)(q13q24)[cp10] 46~47,XY,t(1;2)(q44;q13),inv(3)(p?24.2q29),t(4;9)(q33;q22),t(8;16)(q24.1;q24),t(10;13)(p13;q12)[cp5]
8	44~46,XX,t(1;22)(p32;q11.2),?t(2;20)(q33;q11.2),der(3)t(3;4)(p21;q?31;1)t(3;22)(q21;q13),der(4)t(3;4),?t(9;15)(p22;q22),t(17;18)(q21;q23)[cp15]
9	45~47,Y,del(X)(p22.1),del(1)(q24),?t(8;18)(q24.1;q23),add(9)(q34),?inv(9)(p11q22),del(16)(q?22),add(17)(p11),add(19)(q13),del(20)(q11.2)[cp20]
10	45~46,XY,der(1)t(1;1)(q42;p34.1),der(1)t(1;1)t(1;14)(q24;q11.2),t(3;5)(p23;p11),t(3;10)(q21;q24),t(4;6)(q31.1;p11.2),t(4;16)(q25;p11.2),der(?6)del(6)(p12p21.2)t(6;12)(q?27;?21.2),t(13;16)(q12;q22),der(14)t(1;14)[cp10]
11	46,X,?del(X)(q26q26),inv(3)(p23q?25),inv(7)(p22q22),add(8)(p23),der(8)t(8;9)(p11.2;q22)t(8;13)(q13;q11),der(9)t(8;9),t(10;15)(q26;q12),der(13)t(8;13),del(17)(p11.2p11.2),t(21;22)(q11.2;q13)[cp11] 46,XX,inv(20)(p13q11.2),del(7)(q11.1q11.2),t(14;19)(q11.2;p13.3),del(16)(q23)[2] 46,der(X)del(X)(p11.4)del(X)(q21),der(X)t(X;X)(q26;p11.4),der(5)t(5;9)(q11.2;q32),inv(6)(p23q?13),der(7)t(7;9)(p15;p24),t(7;15)(p12;q15),t(8;12)(q24.1;q22),?t(8;19)(p22;p11),der(9)t(7;9)(p15;p24)t(5;9)(q11.2;q32),t(10;20)(p13;q11.2),del(22)(q13)[3] 37~40,XX,i(1)(q10),-3,?(3;13)(p26;q11),-4,?(4;5)(p14;q33),der(5)t(7;4;5)(q?21;q?33),-6,+7,der(9)add(9)(p24)del(9)(q22),-10,-13,-14,del(15)(q21),-18,add(20)(p11.2),-22,+mar[cp3]

strated. These tumors were particularly aggressive; extensive involvement of the skull base and rapid recurrence were observed. The authors suggested that alterations in the *RB* gene may play a role in the growth of skull base chordomas and that LOH in the *RB* gene may serve as a marker for more aggressive tumors.

Based on the combination of previous findings^{2-4,6,8,9,13,15,18,22,23} and those in the present study, it appears that both lumbosacral and skull base chordomas share a common set of chromosome losses including chromosomes 3, 4, 10, and 13. We have identified additional recurring aberrations in multiple tumors that appear to be frequent in skull base chordomas. These aberrations include isochromosome 1q, gain of chromosome 7, and loss of chromosome 18 in three tumors each. Gain of chromosome 7 has been reported in only one previous lumbosacral chordoma,¹⁸ whereas loss of chromosome 18 has been reported in two cases.^{13,18} To our knowledge, isochromosome 1q has not been reported previously in any chordoma.

In the present study abnormal karyotypes occurred exclusively in recurrent tumors, suggesting that in chordomas chromosome aberrations appear as late events in tumor progression. This may be the case because many types of benign tumors show normal karyotypes, at least until tumor progression.¹⁵ Another possibility is that the presence of many of the aberrations are related to previous radiotherapy. In fact, it is known that exposure to radiation therapy induces cytogenetic aberrations,²⁴ making the interpretation of these tumors more challenging. It is possible, however, to distinguish radiotherapy-induced cyto-

netic artifacts from primary tumor aberrations based on the different responses between normal and tumor tissue. Irradiation of nonmalignant tissues is known to induce many multiclonal balanced chromosome rearrangements that do not show numerical aberrations.²⁴ In contrast, irradiated malignant lesions are characterized more often by monoclonal, frequently unbalanced chromosome rearrangements and frequent clonal evolution.⁵ When multiple clones are found, as in the present cases, there is usually an abnormal clone with characteristic numerical aberrations and unbalanced rearrangements. In this regard, we believe the shared monosomies of chromosomes 3, 4, 10, and 13 indicate a characteristic primary clone that identifies a characteristic “chordoma karyotype” among a background of multiple clones with 46 chromosome and balanced rearrangements.

The molecular cytogenetic technique of SKY was undertaken in three tumors in this series. In two, SKY simply confirmed the results of G banding. In one tumor, however, SKY identified a potentially important unresolved aberration. In the tumor obtained in the patient in Case 6, an add(6)(q23) aberration identified by G banding was refined by SKY to a designation of der(6)t(6;13)(q23;q21). This translocation of chromosomes 6 and 13 (Fig. 1) is important because it results in the loss of the *RB* region (13pter~13q14) by an unbalanced translocation (Fig. 2). If *RB* is important in the progression of chordoma, as suggested by Eisenberg, et al.,¹⁰ then this finding could possibly have prognostic importance. This finding further suggests that other unresolved unbalanced translocations of

Fig. 1. Case 6. Representative G-banded karyotype. Numerical aberrations including monosomy for chromosomes 3, 4, 10, 13, and 18 and trisomy 7 were recurring. Isochromosome 1q (on the right) was the sole recurring structural aberration. Note the subtle aberrations of 1q (on the left), 8p (on the right) and the add(6)(q23) (on the right), which were resolved by SKY (see Fig. 2). Arrows indicate chromosome breakpoints.

chromosome 13 may be present in tumors with complex karyotypes.

The recurring aberrations involving the loss of 1p, as observed here and in previous studies, suggests that an association between the loss of 1p and tumor progression may exist in chordoma. In eight of 13 previously reported chordomas loss of part or all of 1p, was shown^{2,8,13,18} whereas in two additional tumors the breakpoint 1p34 was observed in different reciprocal translocations.^{6,22} In our study three tumors showed clones with the loss of 1p by isochromosome formation, in addition to the primary aberrations of monosomy 3, 4, 10, and 13. This observation indicates that these tumors have acquired an additional secondary structural aberration. Isochromosome 1q formation results in the loss of 1p and the duplication of 1q; therefore, this is an unusual intrachromosomal aberration in which any genes on 1p are lost, and genes on 1q undergo low-level amplification. Isochromosome 1q is frequently found in malignant tumors, as it is a common secondary aberration in breast tumors, Ewing sarcoma, and Wilms tumor.¹⁵ We believe the presence of isochromosome 1q is a new structural aberration that is an additional step in the genomic and chromosomal instability of chordomas.

Skull base chordomas are challenging to manage. Both radical resection and high-dose radiotherapy improve survival rates; however, the unpredictable biological behavior of these tumors remains a limiting factor in treatment.¹ Currently, there is no widely used histological grading

system in the diagnosis of chordomas. To grade chordomas some authors have used a combination of different parameters that include cellular pattern, nuclear atypia, mitosis, necrosis, and vascular invasion.^{12,21} We have used a combination of mitotic activity, degree of nuclear atypia based on pleomorphism and hyperchromatism, and the amount of necrosis as parameters to correlate tentatively the histology and abnormal cytogenetics findings of isochromosome 1q and monosomy 13. Four of the tumors were negative for all histological parameters (Cases 2–4 and 8), whereas in one tumor (Case 7) only nuclear atypia was demonstrated. Four tumors showed two abnormal parameters, in three of which (Case 1, 10, and 11) both increased nuclear atypia and necrosis were shown, whereas in one tumor (Case 9) increased mitosis and necrosis were observed. Increases in all parameters were shown in two tumors (Cases 5 and 6). Interestingly, these latter two tumors with the highest histological grade both had cell lines with isochromosome 1q and -13. Two of the three parameters were abnormal in the third tumor with isochromosome 1q and -13. Although these few cases serve only as a preliminary attempt to correlate histological features and cytogenetics, they suggest that isochromosome 1q and -13 may be associated with higher-grade tumors.

In the present view of tumor development and clonal evolution of chromosome aberrations it is held that malignancy transformation is a multistep process that, in most cases, involves the cooperation of oncogenes, loss of tumor suppressor genes, and alteration of other genes. This

Fig. 2. Case 6. Spectral karyotype of tumor. *Upper:* Spectra-based classification of karyotype shown in display colors. *Lower:* Karyotype of classification-colored chromosomes. Numbers beside chromosomes denote origin of translocated material. Note the identification of subtle translocations of chromosomes 1q and 8p, as well as chromosomes 6q and 13q, which were resolved by SKY. Note the evolution of trisomy 7 to translocations involving chromosomes 11 and 12.

view correlates with the finding of chromosome aberrations in this study, most notably with the deletions (monosomies) and the increasing complexity and instability of the karyotypes. Chordomas as a group show primary monosomies including chromosomes 3, 4, 10, and 13 which are later followed by additional secondary aberrations. The loss of chromosome 13 in most tumors with abnormal karyotypes suggests that the loss of RB is an early and consistent loss in chordomas.¹⁰ Later steps in progression apparently include the formation of isochromosome 1q, the gain of chromosome 7, and loss of chromosome 18. The loss of 1p with concurrent loss of the putative tumor suppressor at 1p36 would potentially be a second tumor suppressor lost in the progression in chordomas. Finally, the loss of chromosome 18 has also been identified as a progressive step associated with the loss of a tumor suppressor gene in the genetic model of colorectal carcinogenesis.¹¹ The identification of a characteristic

karyotype in chordoma could potentially help to identify particularly aggressive tumors as well as supply prognostic information. It remains to be determined by further study if isochromosome 1q and or loss of chromosome 13 will potentially become prognostic markers for tumor progression in chordomas.

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