Cytogenetics as a Tool in the Histologic Subclassification of Chondrosarcomas

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ABSTRACT: Chondrosarcomas are a heterogeneous group of bone neoplasms of which the basic neoplastic tissue is cartilaginous. Frequently the histologic diagnosis and grading of chondrosarcomas is difficult and the histologic appearance does not always reflect the biologic behavior of these tumors. Therefore, it is important to find other parameters that can be of help in the proper diagnosing and grading of these neoplasms. To this end, we attempted to correlate the chromosomal pattern of chondrosarcomas to their histologic subtypes and grades. The cytogenetic analysis of two intermediate-grade chondrosarcomas of bone, and a review of the literature, are presented.

INTRODUCTION
Chondrosarcoma is the second most common primary malignant tumor of bone [1-3]. Histologically, chondrosarcomas are composed of malignant cartilage cells in various stages of maturation, with no evidence of direct osteoid formation. The classic chondrosarcomas of bone are central or peripheral. Variants of the classic type are the clear-cell chondrosarcomas and the mesenchymal chondrosarcomas. The clear-cell chondrosarcoma is the rarest form of chondrosarcoma of bone. It is a slow growing, locally recurring tumor, resembling a chondroblastoma, but with malignant potential. The mesenchymal chondrosarcoma is a rare aggressive variant, and is highly metastatic.

Chondrosarcomas are also found in the soft tissues. They are referred to as extraskeletal chondrosarcomas. Extraskeletal myxoid chondrosarcomas are tumors of low-grade malignancy, which nonetheless are capable of local recurrence and metastasis. These neoplasms occur primarily in the deep soft tissues of the extremities. The mesenchymal chondrosarcoma also has its soft tissue counterpart. Unlike the extraskeletal myxoid chondrosarcoma, it is a highly malignant neoplasm that produces metastases in most of the cases.

Chondrosarcomas are graded I, II, and III based on their cytologic features and number of mitoses. The majority are either grade I or grade II. The histologic diagnosis and grading of chondrosarcomas are difficult. Discrepancies between biologic behavior and histologic appearance are common in these neoplasms. Furthermore the histologic distinction between well-differentiated chondrosarcomas and chondromas can be problematic. Therefore, it is important to find other parameters that can be of help in properly diagnosing and grading these neoplasms. In this study we tried to find a correlation between tumor type, grade, and chromosomal pattern. The cytogenetic analysis of two moderate-grade classic chondrosarcomas is presented here. In addition, a review of the literature is given.

CASE HISTORIES
Case 1
A 54-year-old white male patient, with long-standing pain in the left groin, had on plain X-rays a cartilaginous tumor of the left pubic region that was highly suggestive of malignancy. An open biopsy confirmed the diagnosis of a chondrosarcoma. After thorough staging procedures revealed no metastasis, a left hemipelvectomy was carried out. In the amputation specimen a lobulated, partly encapsulated tumor was present, measuring 15 x 12 x 11 cm. On cross-section the cartilaginous, partly myxoid tumor was attached to the pelvic bones, but the major tumor extension was in the soft tissues towards the bladder. The surgical margins were involved in this area. Histologically the tumor was classified as a grade II chondrosarcoma with marked atypia of the tumor cells, some mitotic activity, and locally cellular areas (Fig. 1).
Cytogenetics in Subclassifying Chondrosarcomas

Figure 1  Tumor tissue of Case 1 composed of atypical cuboidal cells in a chondroid matrix. There are occasional mitotic figures. The tumor has to be classified as grade II chondrosarcoma.

Case 2
A 54-year-old male patient presented with a lytic tumor of the right proximal femur. A core biopsy was taken. This revealed trabecular bone with infiltration of cartilaginous tumor tissue. Several of the tumor cells were binucleated and many revealed plump, hyperchromatic nuclei. Mitotic activity was not observed. A histologic diagnosis of a chondrosarcoma grade II was made. A resection of the proximal femur was performed. The tumor appeared to be localized in the major trochanter, with extension into the surrounding soft tissues. Its maximal diameters were 8 × 6 × 6 cm. On the cut surface the tumor had a glassy appearance compatible with cartilage. Its histology was similar to that of the previous biopsy.

MATERIALS AND METHODS
Fresh representative samples were taken from both tumors. Measurement of cellular DNA content was performed using flow cytometry. Part of the tissue samples was disaggregated and cultured in RPMI 1640 supplemented with 15% FCS, glutamine, and antibiotics. After 16 and 3 days, respectively, the cultures were harvested and chromosome preparations were made according to standard cytogenetic techniques. The chromosomes were G-banded [4].

RESULTS
Case 1
Ten metaphases were analyzed. Eight cells had a hyperdiploid karyotype with an average chromosome number of 52 (range 51-54). Furthermore the tumor cells were characterized by numerous structural abnormalities. Figure 2 shows a representative karyotype of case 1: 51,XY, +Y,dup(2)(p22p23)x2,add(3)(q29)x2,dup(6)(p21p22)x2,+8,del(9)(12),der(17)(8;17)(p21;p13), +18, +der(19)(1;19)(q12;p13),der(20)t(18;20)(q11;p11),add(21)(p13), + add(21)(p13). The two remaining cells were hypotetraploid, containing the double amount of marker chromosomes, as described. The cells presumably originated from a doubling of the hyperdiploid clone. Measurement of cellular DNA content showed a diploid pattern.

Case 2
Cytogenetic investigation of 11 cells revealed a near-diploid karyotype: 47,XY,idic(6)(q13), + 15,dic(16;22)(q11;pll), + 19,der(21)t(16;21)(q11;p11), - 22 (Fig. 3) in six cells. In two other cells an extra copy of chromosome 20 was found. The remaining three cells had different numerical abnormalities. DNA index was 1.0.

DISCUSSION
There are limited data on cytogenetics of chondrosarcomas. Until now 19 cases have been published [5-13]. Fourteen of them were classic chondrosarcomas of bone [10-13], one a clear-cell chondrosarcoma [9], and one a mesenchymal chondrosarcoma ([10] case 15). The remaining three cases were extraskeletal myxoid chondrosarcomas [5-7].

The available data on chromosomal pattern, histological subtypes, and grade are presented in Table 1, together with the present findings. All cytogenetic descriptions are made, and changed according to the ISCN 1991 rules for Cancer Cytogenetics. Three other cases of classic chondrosarcoma presented by Mandahl et al. [10] did not show any clonal ab-
Figure 2  Karyotype of one of the analyzed metaphases of Case I, showing the 51,XY, -Y,dup(2)(p22p23)x2,add(3)(q29)x2,dup(6)(p21p22)x2, +del(9)(q12),der(17)(8;17)(p21; p13), +18,der(19)(1;19)(q12;p13),der(20)(16;20)(q11;p11), add(21)(p13), +add(21)(p13) chromosomal pattern.
Figure 3  The 47,XY,idi(6)(q13), +15,idi(16;22)(q11;p11), +19,der(21)[16;21](q11;p11), -22 karyotype of Case 2.
Table 1  Histologic grade and cytogenetic data of chondrosarcomas (according to the ISCN 1991 for cancer cytogenetics)

<table>
<thead>
<tr>
<th>Case</th>
<th>Reference no.</th>
<th>Grade</th>
<th>Chromosomal pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1</td>
<td>NA</td>
<td>46,XY,t(9;22;15)(q31;q12.2;q25) also found: der(4)(1;4)(q21;p16) and inv(11)(p15q23)</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>NA</td>
<td>46,XY,t(9;22)(q22;q11)/92,XXYY,t(9;22)(q22;q11)x2, + del(1)(p13), - 14</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>NA</td>
<td>46,XY,t(2;13)(q22;p12)</td>
</tr>
</tbody>
</table>

Clear-cell chondrosarcomas

9 1 NA 30,XX,+2,+4,+7,+16,+19,+20,+20,+21,+22

Extraskeletal myxoid chondrosarcomas

46,XY,t(9;22)(q22;q11)/92,XXYY,t(9;22)(q22;q11)x2, + del(1)(p13), - 14, + add(20)(q13), + add(20)(q13), + 22, + 22, + 9-16mar

Mesenchymal chondrosarcomas

10 15 III 95-112,XXYY, + add(1)(q21),x2, - 2,add(4)(p16)x2, - 5, - 6, - 8,add(10)(p11),add(10)(p12), del(11)(p11), - 13,add(15)(p11), + add(15)(p11), + 16, + 17, - 18, - 18, + 19, + 19,add(20)(q13), + add(20)(q13), + 22, + 22, + 9-16mar

Classic chondrosarcomas

10 7 III 79-85,XY, + X, + Y, 1 + 1, + 2, + 3, + 4, + 6, + del(6)(q22), + 7, + 7, + 8, + 8, + 9, + 10, + 11, + 11, + i(12p)(q10), + del(12)(p11p12), + 13, + 14, + 15, + der(15)(11q15)(p11)(p11), + 16, + 17, + 17, + 18, + 18, + 19, + 20, + 21, + 22, + 4-7mar

11 III 38-40,XX,-X,del(5)(q13), - 6, - 10, - 11, - 13, - 14, - 18,add(19)(q13), - 22, - 2-1mar

12 II 44,XY,-2,del(3)(p23),i(6)(p10),add(7)(q22), - 18

13 I 57-60,XY,del(1)(p22p31), + 2, - 4,add(6)(p25), + 7, + 7, + 8, + add(9)(p24), + del(15)(q23),add(16)(p12), + 19, + der(20)(20pter-20q13:7::tq12+1qter), + 6-8mar

14 I 44,XY,del(1)(p11p22),del(1)(11)(q13), - 6, + del(9)(q10), - 12

16 II 43,XY,del(1)(p11p22),del(1)(11)(q13), - 6, + del(9)(q10), - 12, - 22

11 1 I 59,XY, + 2,add(3)(q7),del(4)(p14), + del(4)(q31), - 6, + add(9)(p12),del(9)(p11),del(11)(q23), + 12, + 13,add(14)(p11), + add(15)(p11) - 17, + 19, + 20, + 20, + del(20)(p11) + 21, + add(21)(p11), - 22, + 4mar

12 1 II 52,XY,t(6;12)(q25;q13), + 7, + 8, + 11, + 17, + 19, + 21

13 2 NA° 46,XY,t(12;19)(q13;q13) 1p abnormalities (1p36) among others

This article 1 II 51,XY,Y,dup(2)(p22p23)x2,add(3)(q29)x2,dup(6)(p21p22)x2, + 8,add(9)(q12),del(17)(8;17)(p21;p13), + 18, + der(19)(1;19)(q12;p13),del(20)(18;20)(q11;p11),add(21)(p13), + add(21)(p13)

Abbreviation: NA, data not available.
° Patient had a dedifferentiated chondrosarcoma arising in a central low-grade chondrosarcoma and received chemotherapy prior to cytogenetic investigation.

In more than 80% of Ewing sarcoma a specific translocation, t(11;22)(q24;q12), is found. Ewing sarcoma of bone as well as extraskeletal Ewing sarcoma exhibit this specific translocation, in contrast to chondrosarcomas, where the t(9;22) is found only in the extraskeletal myxoid subtype.

Little is known about the chromosomal patterns of clear-cell chondrosarcomas and mesenchymal chondrosarcomas. From both types only one has been published so far [9], [10], case 15. The clear-cell chondrosarcoma showed a near-haploid chromosomal pattern with no structural abnormalities. A doubling of this pattern was also observed [9]. The lack of structural abnormalities in the clear-cell chondrosarcoma might be explained by the fact that the clear-cell chondrosarcoma is of low-grade malignancy. Furthermore, near haploidy in solid tumors has been associated with a more favorable prognosis [16], which is in agreement with the biologic behavior of this variant.

The mesenchymal chondrosarcoma ([10], case 15) revealed a highly abnormal, hypertetraploid chromosomal pattern with numerous clonal structural abnormalities. Tumor

normalities and therefore are not mentioned here. The chromosomal pattern of case 2 described by Zalupski et al. [13] was too complex to allow complete cytogenetic description.

Cytogenetic analysis of three extraskeletal myxoid chondrosarcomas [5-7] revealed pseudodiploid chromosomal patterns with balanced translocations; in one case a doubling of this pattern was observed as well. In two cases chromosomes 9 and 22 were involved in structural abnormalities. Furthermore Shen et al. [8] performed molecular analysis on a case of extraskeletal myxoid chondrosarcoma and found rearrangement and possible allele loss on 22q11 to 22q12, in addition to allele loss on 10q21 to 10q23 and rearrangement of 10q21.1. Hinrichs et al. [6] proposed that the presence of the oncogene c-sis, which has been localized on 22q11 to 22qter, might play a role in the development of these neoplasms. Abnormalities of chromosome 22 have been described in Ewing sarcoma, Askin tumor, and peripheral neuroepithelioma [14]. Recently Mark et al. [15] reported on the cytogenetics of a case of chondroblastoma in which they found involvement of chromosome 22 in complex translocation.
progression might be related to the genesis of additional chromosomal abnormalities. In this case the complex structural abnormalities and the hypotetraploid chromosome number might explain the malignant behavior of this subtype. In addition, Kreicberg measured the cellular DNA content of 45 chondrosarcomas and correlated this to 10-year survival rates [17]. He found that, regardless of tumor grade, size, and location, patients with diploid DNA content had a better prognosis than patients with tumors with an increase in cellular DNA content.

The cytogenetic analysis of 13 classic chondrosarcomas ([10-13] and the present cases) revealed hypodiploid to hypotetraploid chromosomal patterns with clonal structural abnormalities. Most of the classic chondrosarcomas were either grade I or grade II and had chromosome numbers between 43 and 60. The two grade III tumors ([10], cases 7 and 11) had chromosome numbers of 79-85 and 36-40, respectively. Striking is the finding that in 10 of 13 cases, regardless of histologic grade, chromosome 6 was involved either in a structural abnormality or as loss of a whole copy. In three cases part of the short arm of this chromosome was duplicated. In seven cases the abnormalities led to loss of at least part of the long arm of chromosome 6, with a common loss of the 6q22→6qter region.

Structural rearrangements of chromosome 9 were found in five cases. Breakpoints are gathered in the region 9pter→9q12, whereas breakpoints found in the extraskeletal myxoid chondrosarcoma are in the region 9q22→9q31. In addition, abnormalities of chromosome 9 were found in all of the described grade I classic chondrosarcomas, and in one grade II tumor. No involvement of chromosome 9 was seen in grade III chondrosarcomas. According to these results, there might be a relation between the 9pter→9q12 abnormalities and histologic grade.

In conclusion, the different subtypes of chondrosarcomas reveal some characteristic features in chromosomal pattern. The most obvious one is the translocation between chromosomes 9 and 22, found only in the extraskeletal myxoid chondrosarcomas. In addition, chromosome 6 abnormalities might play an important role in the development of classic chondrosarcomas. Overall we could not find a clear-cut relationship between chromosomal pattern and histologic grade of these neoplasms. However, the complexity of some of the grade I chondrosarcomas might be explained by the fact that some of these low-grade tumors do have the capacity to recur and even metastasize. It is possible that the malignant potential of chondrosarcomas is better reflected by the chromosomal pattern than by the histologic appearance. More research has to be done on this subject, especially as a possible correlation between chromosomal pattern and patient survival rates might be important.

REFERENCES