Cytogenetic analysis of three variants of clival chordoma

Ziv Gil\textsuperscript{a}, Dan M. Fliss\textsuperscript{a,d,*}, Nadia Voskoboinik\textsuperscript{b}, Leonor Leider-Trejo\textsuperscript{c}, Sergey Spektor\textsuperscript{a}, Yuval Yaron\textsuperscript{b,d}, Avi Orr-Urtreger\textsuperscript{b,d}

\textsuperscript{a}Skull Base Surgery Unit, Department of Otolaryngology-Head and Neck Surgery, Tel Aviv Sourasky Medical Center, 6 Weizmann Street, Tel Aviv 64239, Israel
\textsuperscript{b}Genetic Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
\textsuperscript{c}Department of Pathology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
\textsuperscript{d}Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

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Abstract

Chordoma is an uncommon malignant neoplasm derived from remnants of the embryonal notochord. The tumor arises in the sacrococcygeal region in most cases. Cytogenetic information on clival chordomas is scarce due to the low incidence of these tumors. In this study, we applied the G-banding and spectral karyotyping techniques to compare the karyotypes of three variants of clival chordoma: conventional, chondroid, and dedifferentiated. We describe a normal karyotype of a chondroid chordoma and a complex karyotype of a conventional chordoma involving chromosomes 1, 2, 3, 5, 8, 9, 11, 15, 19, 20, and X. The cytogenetic analysis of the dedifferentiated chordoma showed a polyploid complex karyotype of 71–123 chromosomes with double minutes that originated from chromosome 17.

1. Introduction

Chordoma is a rare malignant neoplasm derived from remnants of the embryonal notochord along the axial skeleton. It accounts for 1–4% of malignant osseous lesions, arising most frequently in the sacrococcygeal region and rarely in the sphenop-occipital (clival) or vertebral regions [1]. Clival chordomas are relatively more common among adults between 20 and 40 years of age but may occur at any age, including childhood [2].

Three variants of chordoma have been described: the conventional (classical) type, the chondroid type, and the “dedifferentiated” type, with each one displaying specific microscopic characteristics [1]. The natural history of conventional and chondroid chordomas of the skull base is that of a slow, progressive, and locally aggressive tumor. Metastases are uncommon, occurring in less than 10% of the cases [1]. Piecemeal surgical resection is the mainstay of treatment in these patients because complete excision is rarely possible [3]. The prognosis of patients with conventional or chondroid chordomas depends on the age at occurrence, and 5-year survival varies between 22 and 100%.

Early studies suggested that a better prognosis is associated with the chondroid variant [1]; however, more recent reports showed no significant difference in survival of patients with either conventional or chondroid chordomas [4]. The dedifferentiated chordomas constitute only 1.3–6% of all chordomas [5]. It is a rare and aggressive subtype of chordoma, and it has the biological behavior of a high-grade sarcoma. Dedifferentiated chordoma contains areas of conventional chordoma in association with sarcomatous elements, most frequently resembling malignant fibrous histiocytoma, and rarely fibrosarcoma, high-grade chondrosarcoma, or osteosarcoma [1]. A dedifferentiated chordoma can arise de novo or years later in a patient with recurrent conventional chordoma [6]. The prognosis of patients with dedifferentiated chordoma is poor, and most patients die of local recurrences and metastases within 6–12 months after diagnosis.

Most cytogenetic studies on chordomas have been on those of sacral or spinal origin [7–9]. Approximately half of the cases have been shown to harbor chromosomal aberrations [7], and it has been further suggested that the complex karyotype clones might be associated with high rates of tumor recurrence and poor prognosis [2]. Ten cases of abnormal karyotypes of clival chordoma were reported in the literature, and most of them were recurrent tumors [10]. Comparative genomic hybridization study has showed chromosomal imbalances in five additional cases [8]. In the current study,
we combined conventional G-banding with spectral karyotyping (SKY) analyses to characterize the karyotypes of three variants of clival chordoma, including the first cytogenetic characterization of dedifferentiated chordoma. To the best of our knowledge, this is the first cytogenetic description of dedifferentiated chordoma.

2. Materials and methods

2.1. Clinical and pathological parameters

This study involves three patients who underwent surgery for extirpation of clival chordomas in our institution during 2002–2003. Their family histories were unremarkable. The first case (patient cc01) is a 66-year-old woman who had undergone excision of clival chordoma 2 years before her current referral. A magnetic resonance image of the skull base showed a 2 × 2-cm mass in the inferior part of her clivus. The tumor was completely excised through a midfacial degloving procedure combined with the Le Fort I down fracture approach. The histopathologic examination showed lobules and cords of physaliphorous cells with vacuolated cytoplasm and round nuclei “floating” in a prominent myxoid matrix (Fig. 1A). Immunostaining was positive for cytokeratin and epithelial membrane antigen (EMA). Staining for S-100 protein was negative. These findings supported the diagnosis of conventional chordoma [1].

The second case (patient cc02) involved an otherwise healthy 67-year-old man with a 3 × 4-cm mass that invaded his clivus and sphenoid sinus. The surgical approach was the same as in patient cc01, and complete tumor extirpation was achieved. The histologic examination demonstrated foci of apparent cartilaginous or cartilage-like differentiation in association with areas of conventional chordoma (Fig. 1B). The cells showed immunoreactivity to cytokeratin, EMA, and S-100 protein. The tumor was diagnosed as a chondroid chordoma, a variant of the conventional chordoma.

The third case (patient cc03) was a 47-year-old man who had a large (7.5 × 8-cm) mass in his clival area invading the anterior skull base, brain, and paranasal sinuses. In this case, the subcranial approach was performed to allow access to the intracranial portion of the tumor [11]. Partial excision of the tumor was performed because complete tumor excision was not feasible. Fig. 1C displays the histologic appearance of the tumor, which shows elements of a conventional chordoma in association with areas of high-grade osteosarcoma. The chordoma cells showed immunoreactivity for cytokeratin, EMA, and S-100 protein, and the diagnosis of dedifferentiated chordoma was established [12].

The outcome in this series was based on 18–24 months of clinical and radiologic follow-up. Patients cc01 and cc02 are alive without evidence of residual tumor. The third patient was referred for postoperative adjuvant radiation therapy and died of disease 12 months later.
2.2. Cytogenetic technique

2.2.1. Chromosomal analysis

Fresh samples of the tumors were excised during surgery and submitted for pathologic and cytogenetic analyses. Seven to ten metaphase cells from primary cultures of all tumors were studied using the G-banding technique. A conventional chromosomal analysis was performed on primary short-term cultures. Immediately after tissue removal at surgery, a biopsy from the core of the tumor was mechanically disintegrated and digested for 2 hours with 400 units/mL type II-S collagenase (Sigma, St. Louis, MO). Cell suspension was cultured in RPMI 1640 medium supplemented with 17% fetal calf serum and 2% antibiotics at 37°C in 5% CO₂ for 6–10 days. Cytogenetic analysis was made according to a standard technique, as described previously [13]. Chromosome aberrations were determined according to the International System for Human Cytogenetic Nomenclature, Guidelines for Cancer Cytogenetics [14].

2.2.2. SKY

Conventional cytogenetic analysis was supplemented by the SKY procedure (ASI, Migdal Ha’Emek, Israel). This method enabled visualization of all 24 human chromosomes, with a unique spectral color for each pair of chromosomes. The approach combined Fourier spectroscopy, charge-coupled device imaging, and optical microscopy. Chromosome-specific composite libraries were generated by polymerase chain reaction from flow-sorted human chromosomes conjugated to five different fluorescence dyes (Cy2, SpectrumGreen, Cy3, Texas red, and Cy5). Hybridization was carried out according to the manufacturer’s protocol (ASI) at 37°C in a humid chamber for 72 hours. The metaphases were captured and analyzed using the SD200 system (ASI) and triple filter (SKY CUBE; ASI). To generate a classified spectral karyotype, the acquired spectral image was analyzed using SKYVIEW software (ASI).

3. Results

Cytogenetic analysis using G-banding of the first case (patient cc01, diagnosed as recurrent conventional chordoma of the clivus), suggested an abnormal karyotype (Fig. 2). Confirmation of the cytogenetic data was performed using the SKY technique and it showed a complex karyotype (Fig. 3). The tumor karyotype of patient cc01 based on the G-banding and SKY analyses was as follows: 45–46,X,del(X)(q23)[3],t(2;9)(p23;p24)[5],t(3;19)(q26;q13)[4],t(5;15)(p13;q26)[5],+der(8)[3][cp7]. The second case (patient cc02),

![Fig. 2. G-banding karyotype of a conventional chordoma from patient cc01: 46,X,del(X)(q23),t(1;11)(q21;q23),t(2;9)(p23;p24),−3,t(5;15)(p13;q26),−20, +mar. The arrows indicate the abnormal chromosomes. Translocation (1;11), −3, and −20 were nonclonal abnormalities.](image-url)
diagnosed as chondroid clival chordoma, had a normal karyotype (data not shown). The third case (patient cc03), diagnosed as dedifferentiated chordoma, demonstrated a polyploid complex karyotype with multiple numerical and structural chromosomal aberrations: 71,XY,+X,+1,+1,+2,+2,+5,+6,+6,+7,+7,+8,+9,+11,add(11)(p15),+12,+14,+14,+16,+7mar,3dmin (Fig. 4). Combined G-banding and SKY analysis of this case revealed a complex karyotype of 71–123 chromosomes with double minutes. As determined by SKY, the double minutes originated from chromosome 17 (Fig. 5). The clinical and cytogenetic findings are summarized in Table 1.

4. Discussion

Cytogenetic information on clival chordomas is scarce due to the low incidence of this tumor. Sandberg and Bridge [7] have reported 29 cytogenetically abnormal cases of chordoma. Fifteen of these cases involved the clivus. Previously reported frequent chromosomal aberrations found in chordomas involved the loss of chromosomes 3, 4, 10, and 13, as well as structural rearrangement of chromosome arms 1p and 21q22 [7–9].

In this study, we described the cytogenetic features of three variants of clival chordoma. We found a normal karyotype in one case of chondroid chordoma, while the conventional type of chordoma described herein had a complex karyotype involving numerical and structural aberrations of chromosomes 1, 2, 3, 5, 8, 9, 11, 15, 19, 20, and X. The loss of chromosome 3 as part of a complex karyotype was described previously in 10 cases of sacral chordoma [7–9, 15–17], with one of these cases also showing loss of chromosome 20, similar to that found in our patient cc01. Gibas et al. [18] described breakpoints of 5p13, 9p24, and 19q13 in a case of sacral chordoma. A breakpoint of 11q23 was reported in another case of sacral chordoma [19], and a breakpoint of 1q21 was found in two other cases of sacral chordoma [16,18] and in one case of familial clival chordoma [20]. Breakpoints of chromosomes 2q13, 3q26, 15q26, and Xq23 have not been described previously.

The involvement of chromosome arm 11q23, as described in patient cc01, has been documented in a wide variety of soft-tissue tumors, as well as in breast, uterus, bladder, and ovarian cancers [21]. A tumor suppression gene localized to a 700-kb cloned region within the 5-cM interval of loss of heterozygosity (LOH) on the same chromosome arm was reported recently [22]. The high incidence of LOH for 11q23 raises the possibility that at least one gene in this region is responsible for tumorigenesis of a variety of neoplasms including chondroid chordoma.

In this work, we also described the first karyotype of a dedifferentiated chordoma. This tumor showed elements of a conventional chordoma in association with areas of high-grade osteosarcoma (Fig. 1C). Cytogenetic analysis revealed a polyploid complex karyotype of 71–123 chromosomes with double minutes. Interestingly, polyploid karyotypes of 48–97 chromosomes with double minutes were also described in four other cases of high-grade osteosarcoma [23,24]. Such numerous structural abnormalities involving additions of unbalanced translocations or deletions are frequently found in high-grade osteosarcomas [25–28]. In contrast, of all the previously described chordoma karyotypes, only one involved a polyploid karyotype with 72 chromosomes [18], and double minutes were never described before
in this tumor. Using the SKY technique, we determined that the origin of the double minutes was in chromosome 17, although we did not determine its specific location. Few genes involved in tumorigenesis are located on chromosome 17, among them TP53, BRCA1, ERBB2, and NF1. Further studies are required to identify the exact area on chromosome 17 from which the double minutes originate.

The growing complexity of the karyotype found in dedifferentiated chordoma is in keeping with the presently accepted view that a multistep process involving oncogenes, loss of tumor suppression genes, and other genetic alterations are cooperatively involved in anaplastic transformation [29].

The pathogenetic evolution of the dedifferentiated chordoma and its relation with simple chordomas is unknown. It may evolve from a common progenitor stem cell (i.e., the divergent theory) or from separate clones with distinct differentiation pathways (i.e., the collision tumor or convergent theory). If two clones are involved, one clone differentiates into a chordoma and the other (which is deficient of any differentiation potential) evolves into a high-grade sarcoma [30]. Alternatively, it was suggested that the high-grade components of the tumor might emerge directly from the more indolent chordoma by a mechanism of dedifferentiation [31]. The exact mechanism of dedifferentiation is not clear; it has been postulated that progression toward an anaplastic component could be regarded as a failure of differentiation rather than a reversal of differentiated cell to an embryonic undifferentiated cell [32].

Our sample analysis of the dedifferentiated variant most probably included both the chordoma and osteosarcoma components. It is therefore most probable that the cytogenetic characteristics of the tumor represent both these components. Ideally, in future studies, grossly differing regions of low- and high-grade tumor should be sampled and analyzed separately to improve our understanding of the mechanism of tumor progression and evolution.

To date, the extent of the cytogenetic data is too limited to furnish precise information on the evolutionary process of a dedifferentiated chordoma. Genetic characterization of different chordoma variants should provide further information useful for the understanding of the pathologic evolution of high-grade sarcoma components from the more indolent...
chordoma cells. Additional studies are also required to determine whether the presence of a polyploid karyotype with or without double minutes can differentiate simple chordomas from dedifferentiated chordomas.

Half of the chordoma cases reported in the literature harbor a normal karyotype [7]. In our study, one of the two conventional chordomas also showed a normal karyotype. Sawyer et al. have reported a normal karyotype in all primary clival chordomas, whereas all recurrent tumors showed chromosomal aberrations [10]. It was therefore postulated that chromosomal aberrations occur late in the progression of chordoma, predominantly in recurrent cases [10].

In conclusion, we suggest that conventional clival chordomas can display a nearly diploid karyotype. These tumors are associated with a good prognosis following complete extirpation of the tumor. In contrast, the dedifferentiated

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**Table 1**

Clinical and cytogenetic features of the patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/gender</th>
<th>Tumor size (cm)</th>
<th>Tumor type</th>
<th>Follow-up</th>
<th>Karyotype</th>
<th>Cells analyzed</th>
<th>Abnormal karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>66/F</td>
<td>2 × 2</td>
<td>Conventional chordoma</td>
<td>FOD</td>
<td>45–46.X.del(X)(q23)[3],t(2;9)(p23;24)[5],t(13;19)(q13)[4],t(5;15)[p13;q26][5],d[8][cp7]</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>02</td>
<td>67/M</td>
<td>3 × 4</td>
<td>Chondroid chordoma</td>
<td>FOD</td>
<td>46.XY</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>03</td>
<td>47/M</td>
<td>7.5 × 8</td>
<td>Dedifferentiated chordoma</td>
<td>DOD</td>
<td>71.XY, +X, +1, +1, +2, +2, +5, +6, +6, +7, +7, +8, +9, -11, add(11)(p15), +12, +14, +14, +16, +7mar, 3dmin</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

*Abbreviations: F, female; M, male; DOD, died of disease; FOD, free of disease.*
chordoma harbors a polyplid complex karyotype and is associated with aggressive biological behavior and a poor prognosis. Most of the cytogenetic data related to chordomas are derived from tumors involving the sacral and vertebral areas. Whether similar genetic patterns exist in chordomas of the clivus awaits clarification.

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References

[13] Gil Z, Fliss DM, Voskoboinik N, Trejo-Leider L, Khatif A, Yaron Y, Orr-Urtreger A. Two novel translocations, t(2;4)(q35;q31) and t(X;12) (q22;q24), as the only karyotypic abnormalities in a malignant peripheral nerve sheath tumor of the skull base. Cancer Genet Cytogenet 2003;145:139–43.