Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jor

Original Article

Abnormal karyotypes in osteochondroma: Case series and literature review

Aaron W. James^a, Carlos A. Tirado^a, Benjamin Levine^b, Sarah M. Dry^{a,*}^aDepartment of Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA^bDepartment of Radiology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:

Received 19 September 2013

Accepted 29 December 2013

Available online 30 January 2014

Keywords:

Osteochondroma

Karyotype

Exostosis

Cartilage neoplasm

EXT1

ABSTRACT

Background: Osteochondroma is the most common benign bone tumor. However, there are infrequent studies karyotyping solitary osteochondromas.**Methods:** Retrospective review of the University of California, Los Angeles pathology database was performed for karyotype analyses (N = 522 specimens).**Results:** Two previously undescribed karyotypes were identified. First, was a karyotype showing paracentric inversion of chromosome 7. Second, was a karyotype showing monosomy 3, 6 and 13.**Conclusions:** Abnormal karyotypes may be more frequently encountered in osteochondroma than previously understood. However, the clinical significance of these abnormalities are yet unknown.

Copyright © 2014, Professor P K Surendran Memorial Education Foundation. Publishing Services by Reed Elsevier India Pvt. Ltd. All rights reserved.

1. Introduction

Osteochondroma is a benign bony protrusion covered by a cartilaginous cap, commonly involving the long bones. Osteochondroma is the most common bone tumor, with an estimated incidence of 35% of benign bone tumors and 8% of all bone tumors, although these numbers may be an underestimate.¹ Osteochondromas, although benign, can cause significant local symptoms in a minority of cases including nerve impingement, mechanical obstruction, pseudoaneurysm of adjacent vessels, and infarction or fracture of the tumor itself. Osteochondromas have been identified in skeletons dated as

far back as 11th century Croatia,² 2nd century Poland,³ and even 3rd century BC Britain.⁴ Despite their historical and current day prevalence, accompanying morbidity and potential for malignant transformation, the underlying biology and etiology of osteochondromas is still poorly understood.

Osteochondromas may occur as either sporadic solitary lesions or as multiple lesions as part the autosomal dominant, hereditary multiple exostoses (HME).^{5,6} The histologic appearance of sporadic or multiple/hereditary osteochondromas is identical. The genetic loci for HME have been identified (all of which are also involved in sporadic osteochondroma): 8q24.1 (EXT1), 11p11~12 (EXT2), and 19p (EXT3).^{7–9} The identification of EXT1-3 polymerase mutations have made it clear that

* Corresponding author. Department of Pathology & Laboratory Medicine, University of California, Los Angeles, David Geffen School of Medicine, 10833 Le Conte Ave., 13-145 CHS, Los Angeles, CA 90095, USA. Tel.: +1 310 206 0382.

E-mail addresses: SDry@mednet.ucla.edu, aaronwjames1@gmail.com (S.M. Dry).

0972-978X/\$ – see front matter Copyright © 2014, Professor P K Surendran Memorial Education Foundation. Publishing Services by Reed Elsevier India Pvt. Ltd. All rights reserved.
<http://dx.doi.org/10.1016/j.jor.2013.12.015>

osteochondromas represent true cartilaginous tumors, rather than developmental malformations.

Collectively, there has been little interest in the cytogenetic characterization of sporadic osteochondromas, and karyotypes are not routinely obtained in solitary osteochondromas. Bridge et al, described the karyotype of 34 osteochondroma specimens of which 29% showed clonal karyotypic abnormalities.¹⁰ Feely et al later expanded this dataset to describe the karyotype of 37 osteochondroma specimens of which up to 51% showed either random or clonal karyotypic abnormalities.¹¹ The most frequent chromosomes involved were chromosomes 8, 6 and 12 in descending order of frequency. The majority of chromosome 8 abnormalities involved loss or rearrangement of 8q24.1 (EXT1), and chromosome 11 abnormalities typically involved 11p11-12 (EXT2).¹⁰ However, not all osteochondromas have detectable EXT mutations. For example, Bridge et al found 8q22-24 (EXT1) aberrations in only 33% of specimens examined, while Feely et al found 8q24.1 loss by FISH in 79% of cases.^{10,11} In aggregate, these data suggest that EXT mutations are the main associated cytogenetic alteration in osteochondroma, but also that other, as yet unknown, cytogenetic aberrations may also predispose for the formation or growth of osteochondroma.

Other chromosomal aberrations have been identified in osteochondromas. For example, several other breakage points include 12q13, 2p21, 6p25 and 3q27.¹⁰ Of these, 12q13-15 is found in both benign and malignant cartilage neoplasms and localizes to the major component of cartilage matrix, Type II collagen.^{10,12} However, it is clear that in general benign cartilage tumors have less complex karyotypes than their malignant counterpart (chondrosarcoma).¹¹ Thus, there are multiple cytogenetic aberrations that accompany, and possibly incite, formation of osteochondroma. Undoubtedly, the continued study of sporadic osteochondromas will find of cytogenetic aberrations of biological relevance to improve our understanding of this common bone tumor.

To this end, we examined our pathology database of 522 osteochondromas to identify any previously undocumented cytogenetic abnormalities. Our hypothesis was that as yet unidentified cytogenetic aberrations may be present in our database of specimens, and that identification of new chromosomal changes may yield profitable future insight into the genetic bases for this common tumor.

2. Materials and methods

2.1. Database search

Computerized search of the University of California, Los Angeles (UCLA) pathology database from 2000 to 2012 was performed for any karyotype analysis, using the search terms 'osteochondroma(s), exostosis(es). Clinical data was obtained including patient age, gender, and pertinent medical, surgical, radiographic and pathologic records.

2.2. Chromosome analysis

A G-banded chromosome study was performed using standard cytogenetic techniques. Briefly, two unstimulated

cultures were set up in RPMI 1640 medium enriched with 20% fetal calf serum, giant cell tumor conditioning media, L-glutamine, and antibiotics (penicillin and streptomycin). The cells were cultured for 24 and 48 h in a humidified environment with 5% CO₂ in a 37°C incubator until harvest.

Before harvesting, the cultures were treated with Colcemid (25 mL) for 16e18 h. Soon after, the cells were exposed to hypotonic solution (0.075 mol/L KCl) and fixed with methanol/acetic acid (3:1). The slides were prepared and stained using a G-banding (Trypsin-Giemsa-Wright) technique. Up to twenty metaphases were analyzed and karyograms were prepared using the CytoVision computer assisted karyotyping system (Applied Imaging, Santa Clara, CA, USA). The karyotypes were described according to the International System for Human Cytogenetics Nomenclature.¹³

3. Results

Karyotypes were infrequently ordered for osteochondroma (3/522 patients, 0.57%) in records dating back 12 years. Of these, two abnormal karyotypes were identified. The first was a 22 year old, otherwise well female with an asymptomatic, palpable right rib mass noticed for 3 weeks duration. No personal or family history of bone tumors was elicited. Computerized tomography (CT) of the chest demonstrated an expansile, partially ossified, 3.4 cm mass arising from the anterolateral aspect of the right first rib (Fig. 1a,b). Bone scan showed intense hypermetabolic focus in the area of the mass, with no significant uptake elsewhere. Biopsy showed hypocellular lobular cartilage adjacent to trabecular bone with marrow elements, suggestive of osteochondroma. Surgical excision was performed, showing a large, pedunculated lesion arising from the resected rib (Fig. 1c). Histological analysis of the resection specimen showed hypocellular lobular cartilage with overlying periosteal fibrous tissue, and with underlying endochondral ossification, and trabecular bone with bone marrow (Fig. 1d), confirming the diagnosis of osteochondroma. No cytologic atypia was observed. Karyotyping was performed from biopsy tissue as the size of the lesion was concerning for malignancy. Karyotyping demonstrated monosomy of chromosomes 3, 6 and 13, additional material of unknown origin on 4q28 and 3 marker chromosomes in all metaphase cells examined. The karyotype was described as: 46,XX,-3,add(4)(q28),-6,-13,+3mar[10] (Fig. 1e).

The second patient was an otherwise well, 37-year-old female who presented with a palpable mass noticed over her left knee for the past 8 months. No personal or family history of bone tumors was elicited. CT of the leg demonstrated a well corticated, 2 cm osseous protrusion along the anteromedial aspect of the tibial tubercle, with medullary and cortical continuity with the underlying bone (Fig. 2a). Surgical excision was performed, and the resection specimen was received in fragments. As malignancy could not be excluded, karyotyping was performed as well as routine processing for histology. Based on the typical histologic appearance, a pathologic diagnosis of osteochondroma was rendered (Fig. 2b,c). Typical findings of osteochondroma were appreciated, including low-grade cartilage, with an outer fibrous periosteum, and underlying bone with marrow cavity. No cytologic atypia was

Fig. 1 – Osteochondroma with monosomy 3, 6, and 13. (a, b) Coronal and sagittal CT images of chest, demonstrating a bony exostosis arising from the anterolateral aspect of the first right rib (white arrows). Note that the cortex and medullary cavity of the mass is continuous with the parent bone. (c) Gross appearance after resection, with accompanying portion of first rib. (d) Histologic appearance of resection specimen, demonstrating typical features of an osteochondroma, including low-grade lobular cartilage (asterisk) with underlying endochondral ossification, trabecular bone, and marrow elements (black arrows) (H&E 4×, respectively). (e) Routine chromosomal analysis detected an abnormal female chromosome complement in all cells with monosomy of chromosomes 3, 6 and 13, with additional material of unknown origin on 4q28 and 3 marker chromosomes of unknown origin. The karyotype was interpreted as: 46,XX,-3,add(4)(q28),-6,-13,+3mar[10].

observed. Karyotyping of the lesion showed a paracentric inversion of chromosome 7 in 15/20 cells. Three of these cells showed in addition a reciprocal translocation between the long arms of chromosomes 8 and 15 (Fig. 2d). This karyotype was described as: 46,XX,inv(7)(q22q32)[12]/46,idem,t(8;15)(q13;q11.2)[3]/46,XX[5].

The third patient was an otherwise well, 18-year-old female with a large, pedunculated tumor of the right distal medial femoral condyle. Surgical excision was performed, and the resection specimen measured 4.5 cm in maximum dimension. Due to the tumor size and a concern for malignancy, karyotyping was performed as well as routine processing for histology. Based on the typical histologic appearance, a pathologic diagnosis of osteochondroma was rendered. Karyotyping of the lesion showed a normal female karyotype, 46,XX[15].

4. Discussion

We present two previously undescribed karyotype abnormalities in osteochondroma, including paracentric inversion of chromosome 7, and a case of monosomy 3, 6, and 13. In addition, one case showed a more typical translocation of

osteochondroma involving the long arm of chromosome 8. Previously reported karyotypic abnormalities involving chromosome 7 include deletion 7q22q36 (involving the same locus as in our case of the paracentric inversion).^{10,11} Karyotypic abnormalities of chromosome 6 are common in both benign and malignant cartilage neoplasms.^{11,14} Feely et al described recurrent aberrations in chromosomes 3 and 6 (including monosomy 3 in a single case).¹¹ The biologic, clinical and prognostic significance of these karyotype abnormalities is unknown – although both patients were recurrence free at over 5 and 10 years, respectively.

There are several limitations to the present case study. First, although a large number of osteochondromas have been diagnosed at our institution, karyotyping is not frequently observed. In fact, karyotyping was only performed if the diagnosis was uncertain at the time of specimen processing. Thus, the incidence of 66% of cytogenetic aberrations (66%, or 2/3 specimens) may be an overestimate – although it is in agreement with other studies. Second, the present study uses karyotyping as the only screening tool for cytogenetic abnormalities. Of course, there are many more sensitive methods for analysis, including targeted FISH analyses, PCR based sequencing of specific genes, or chromosomal microarray. In fact, CGH-arrays have already been used to examine cases of

Fig. 2 – Osteochondroma with paracentric inversion of chromosome 7. (a) Axial CT image of the lower leg, demonstrating a bony exostosis arising from the anteromedial aspect of the proximal tibia (white arrows). Note that the cortex and medullary cavity of the mass is continuous with the parent bone. **(b, c)** Histologic appearance of resection specimen, demonstrating low-grade lobular cartilage (black asterisks), with overlying perichondrium (white asterisk), and underlying zone of endochondral ossification (H&E 4× and 10×, respectively). **(d)** Routine chromosomal analysis detected an abnormal female chromosome complement in which 15 of 20 cells exhibited a paracentric inversion of chromosome 7. A subclone also exhibited a balanced, reciprocal translocation between the long arms of chromosomes 8 and 15. Final karyotype interpreted as: 46,XX,inv(7)(q22q32)[12]/46,idem,t(8;15)(q13;q11.2)[3]/46,XX[5].

multiple osteochondromas.¹⁵ A similar approach to sporadic osteochondromas may likewise lead to additional insights.

In summary, the frequency of cytogenetic abnormalities in osteochondroma may be higher than appreciated. Although cytogenetic analysis of osteochondromas is not commonly performed, it is interesting to speculate on possible future uses. These include: 1) use of cytogenetic analyses as an adjunctive tool for the differentiation of benign and malignant cartilage tumors, and 2) use of cytogenetic analyses in the prognostication for risk of malignant transformation in osteochondroma. In sum, systematic cytogenetic analyses of osteochondromas may elucidate further inciting genetic events for this common entity.

Conflicts of interest

All authors have none to declare.

Acknowledgments

The authors thank Ms. L. Chang for her excellent technical assistance.

REFERENCES

1. Unni K. *Dahlin's Bone Tumors General Aspects and Data on 11,087 Cases*. 5th ed. Philadelphia: Lippincott–Raven; 1996.
2. Slaus M, Orlic D, Pecina M. Osteochondroma in a skeleton from an 11th century Croatian cemetery. *Croat Med J*. 2000 Sep;41:336–340.
3. Gladyskowska-Rzeczycka J, Urbanowicz M. Multiple exostoses in a skeleton from a prehistoric cemetery of the former population of Pruszcz Gdanski. *Folia Morphol Warsz*. 1970;29:317–329.
4. Chamberlain AT, Rogers S, Romanowski CA. Osteochondroma in a British neolithic skeleton. *Br J Hosp Med*. 1992 Jan 9–22;47:51–53.
5. Schmale GA, Conrad 3rd EU, Raskind WH. The natural history of hereditary multiple exostoses. *J Bone Joint Surg*. 1994 Jul;76:986–992.
6. Potocki L, Shaffer LG. Interstitial deletion of 11(p11.2p12): a newly described contiguous gene deletion syndrome involving the gene for hereditary multiple exostoses (EXT2). *Am J Med Genet*. 1996 Mar 29;62:319–325.
7. Cook A, Raskind W, Blanton SH, et al. Genetic heterogeneity in families with hereditary multiple exostoses. *Am J Hum Genet*. 1993 Jul;53:71–79.
8. Wu YQ, Heutink P, de Vries BB, et al. Assignment of a second locus for multiple exostoses to the pericentromeric region of chromosome 11. *Hum Mol Genet*. 1994 Jan;3:167–171.

9. Le Merrer M, Legeai-Mallet L, Jeannin PM, et al. A gene for hereditary multiple exostoses maps to chromosome 19p. *Hum Mol Genet.* 1994 May;3:717–722.
10. Bridge JA, Nelson M, Orndal C, Bhatia P, Neff JR. Clonal karyotypic abnormalities of the hereditary multiple exostoses chromosomal loci 8q24.1 (EXT1) and 11p11-12 (EXT2) in patients with sporadic and hereditary osteochondromas. *Cancer.* 1998 May 1;82:1657–1663.
11. Feely MG, Boehm AK, Bridge RS, et al. Cytogenetic and molecular cytogenetic evidence of recurrent 8q24.1 loss in osteochondroma. *Cancer Genet Cytogenet.* 2002 Sep;137:102–107.
12. Hecht JT, Hogue D, Wang Y, et al. Hereditary multiple exostoses (EXT): mutational studies of familial EXT1 cases and EXT-associated malignancies. *Am J Hum Genet.* 1997 Jan;60:80–86.
13. Schaffer LG, McGowan-Jordan J, Schmid M. In: Karger S, ed. *ISCN 2013: An International System for Human Cytogenetic Nomenclature.* 2013.
14. Bridge JA, Bhatia PS, Anderson JR, Neff JR. Biologic and clinical significance of cytogenetic and molecular cytogenetic abnormalities in benign and malignant cartilaginous lesions. *Cancer Genet Cytogenet.* 1993 Sep;69:79–90.
15. Szuhai K, Jennes I, de Jong D, et al. Tiling resolution array-CGH shows that somatic mosaic deletion of the EXT gene is causative in EXT gene mutation negative multiple osteochondromas patients. *Hum Mutat.* 2011 Feb;32:E2036–E2049.