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Lead Article

Genome profiling of chondrosarcoma using oligonucleotide array-based comparative genomic hybridization

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Abstract Chondrosarcomas of the bone are malignant hyaline cartilage-forming tumors with an annual incidence rate of 3.6% of all primary bone malignancies in the United States. Specimens of 25 chondrosarcomas (10 grade I, 9 grade II, 1 grade III, and 5 dedifferentiated) from 23 patients were collected from the Department of Pathology at the University Hospital at UMDNJ-New Jersey Medical School from 1996 to 2007. Array-based comparative genomic hybridization (array-CGH) studies were performed on frozen tumor specimens. Recurrent deletions observed in at least in six tumors were 5q13.2, 5q14.2~q21.3, 6q12~q13, 6q16~q25.3, 9p24.2~q12, and 9p21.3. There was a statistically significant association between high-grade tumor (grade III and dedifferentiated) and the recurrent genetic deletions at 5q14.2~q21.3, 6q16~q25.3, 9p24.2~q12, and 9p21.3. There is consistency between increased levels of aneuploidy and the progression of chondrosarcoma from lower to higher grades.

1. Introduction

Chondrosarcoma of the bone is a malignant hyaline cartilage-matrix forming tumor that affects both males and females equally with a peak incidence around the fifth decade. It is the third most common primary malignant tumor of bone, after multiple myeloma and osteosarcoma [1]. It is subclassified as central or peripheral, depending on location: central if arising from within the medullary cavity of bone and peripheral if arising from the surface of bone. Prognostic factors depend on histological grading, with a 5-year survival of 89% for grade I tumors and ~50% for grade II and III. Dedifferentiated chondrosarcomas have a dismal 1-year survival of $<10\%$ [1]. The majority of grade I and II tumors usually grow slowly and respond very well to surgical intervention, but treatment options for grade III and dedifferentiated tumors are often limited, because these tumors are mostly resistant to chemotherapy and radiotherapy [2].

The cytogenetic characteristics of chondrosarcoma have been extensively studied. Most studies, however, have used conventional cytogenetic methods, such as karyotyping or classical comparative genomic hybridization (CGH) [3–13]. To date, two array-based CGH studies have been conducted using bacterial artificial chromosome- (BAC-based) array systems [3,4]. The advances in array-CGH technology in recent years have resulted in the replacement of BAC arrays with oligonucleotide based arrays, because the latter have a much higher resolution than do BAC arrays [14]. Thus far, there has been no reported study of chondrosarcomas using oligonucleotide-based arrays.

The objective of this study was to perform array-CGH analysis on archived chondrosarcoma tumors samples and to correlate genetic aberrations to the tumor grade.

2. Materials and methods

2.1. Study group

The study was performed after Institutional Review Board approval. Twenty-five archived frozen specimens of

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chondrosarcomas from 23 patients were collected from the Department of Pathology at University Hospital at UMDNJ-New Jersey Medical School from 1996 to 2007. These specimens were either snap-frozen or stored in optimal cutting temperature compound. Pathological examination of all tumors were done in conjunction with radiological correlation. There were 10 grade 1, 9 grade II, 1 grade III, and 5 dedifferentiated tumors. All except one (grade III, juxtacortical) were central chondrosarcomas. Two tumors were originally classified as cartilage tumors of uncertain malignant potential but upon re-review were classified as grade I chondrosarcomas. Of the five dedifferentiated chondrosarcomas, two showed only dedifferentiated component in frozen material, two showed well differentiated component only (histologically grade II) in the frozen material, and in one case two components were frozen separately with availability of both well-differentiated (grade II) and dedifferentiated components from the same tumor (samples 12a and 12b). Of the grade II tumors, one was a recurrent myxoid chondrosarcoma from the same patient (samples 23 and 2). Table 1 lists clinical and pathological characteristics.

2.2. DNA isolation

Genomic DNA from tissue specimens was extracted according to the manufacturer's instructions using a Gentra Systems Puregene tissue kit (Qiagen, Valencia, CA).

Table 1
Clinical and pathological characteristics for 23 chondrosarcoma patients

Sample	Age, yr	Sex	Grade	Site	Year of diagnosis
1	51	F	II	Pelvis	2000
2[a]	37	M	II	Pelvis	2006
3	47	F	I	Humerus	2000
4	16	M	II	Shoulder	1999
5	35	F	I	Pelvis	1999
6[b]	55	M	Dedifferentiated	Femur	2002
7	82	F	Dedifferentiated	Humerus	2003
8	58	M	Dedifferentiated	Femur	1999
9	34	F	II	Femur	1997
10[b]	62	M	Dedifferentiated	Femur	1996
11	50	F	I	Humerus	2000
12a[a]	59	F	II	Pelvis	1997
12b[a]	59	F	Dedifferentiated	Pelvis	1997
14	27	M	I	Femur	1996
15	59	F	I	Radius	1997
16	33	F	I	Femur	1998
17	19	M	II	Fibula	2001
18	68	F	I	Humerus	2000
19	70	M	III	Humerus	2002
20	40	M	I	Femur	2003
21	31	F	II	Pelvis	2002
22	59	F	I	Femur	2000
23[a]	34	M	II	Femur	2003
24	50	F	II	Sacrum	2002
25	43	F	I	Femur	2002

[a] Tumor samples 2 and 23 are from the same tumor in a single patient, as are samples 12a and 12b.

[b] Only the well-differentiated frozen component of the tumor was analyzed.

2.3. Array-CGH experiments

Custom 4 × 44 K arrays from Agilent Technologies were used for array-CGH experiments. Both the array and experimental procedures were as previously described [15]. Sex-matched commercial DNA samples (Promega, Madison, WI) were used as the reference DNA. Microarrays were scanned on an Agilent G2565BA scanner. Data from scanned images were extracted by Agilent Feature Extraction software version 9.5, and then imported into Agilent CGH Analytics version 3.5 software for further analysis. During data analysis with the CGH analytics software, the statistical algorithm was ADM-1, the sensitivity threshold was 6.0, and the moving average window was 2 Mb.

A copy number change in a particular locus was determined according to three criteria. These were positive call by the software, presence of at least seven consecutive probes pointing out the same direction, and 1.5-fold average difference in the test DNA relative to the reference normal DNA. Only changes that met all three criteria were scored as positive. The coordinates are based on NCBI 35 Genome Build (HG17, May 2004)

3. Results and discussion

At least one genetic aberration was detected in every tumor specimen. The results for all samples at the 550-band level are summarized in Figure 1. The breakpoints of genetic aberrations for each sample are presented in supplementary Table 1 (available at http://www.sciencedirect.com/science/journal/01654608).

Several recurrent aberrations were identified. The criteria used for determining recurrent regions were the size of the genetic aberration and the number of the tumors having that genetic aberration. Only aberrations larger than 2 Mb and observed in at least six tumors were scored as recurrent genetic aberration. The observed recurrent deletions were 5q13.2, 5q14.2~q21.3, 6q12~q13, 6q16~q25.3, 9p24.2~q12, and 9p21.3. (Table 2).

The recurrent aberrations seem to be accumulated in tumors of higher grade. Although the sample size is relatively small, there was a statistically significant association between grade III and dedifferentiated tumors and the recurrent genetic aberrations at loci 5q14.2~q21.3, 6q16~q25.3, 9p24.2~q12, and 9p21.3. (Table 2).

In general, the present results are concordant with previous studies. All of the recurrent aberrations identified in this study have been reported previously [3–13]. This is not surprising, considering the range of numerical and structural cytogenetic aberrations seen in most malignant neoplasms. On the other hand, in the present cohort we did not observe previously described recurrent amplification at 8q24.12~q13 [4] or chromosome 12 amplifications [3].

One of the most commonly deleted regions in our study group involved chromosome 9, which was seen in one third