Genetic imbalances in benign bone tumors revealed by comparative genomic hybridization*

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There have been no reports on choromosomal aberrations of benign bone tumors revealed by comparative genomic hybridization (CGH). CGH analysis of benign tumors may be useful in understanding the mechanism of tumorigenesis with comparisons to malignant tumors. There were 4 tumors (2 enchondromas, one chondromyxoid fibroma, and one osteoid osteoma) and 8 tumor-like conditions (4 aneurysmal bone cysts (ABCs), one eosinophilic granuloma, one fibrous dysplasia, one solitary bone cyst, and one Rosai-Dorfman disease) available for analysis. One of 2 enchondromas and one of 4 ABCs exhibited rapid growth. Six lesions showed chromosomal aberrations, while 6 others did not. The most frequent aberrations were the loss of a whole chromosome-19 in 6 cases, the loss of chromosome-arm 22q in 4 cases, and the loss of chromosome-arm 17p in 3 cases. Gains were seen in 13q21 in 2 cartilaginous tumors and at 12q15-q21 in eosinophilic granulomas. Therefore, in benign bone tumors or tumor-like lesions, chromosomal aberrations are not frequent; however, some clear tendencies of clustering of aberrations can be observed.

Key words: genetic imbalance, genomic hybridization, benign tumors, chromosomal aberrations

Comparative genomic hybridization (CGH) is a relatively new technique for a genome-wide screening of DNA sequence copy number changes. In recent years, there have been several reports of CGH analysis of osteosarcoma [4, 11, 19, 21], Ewing tumor [10, 22], and chondrosarcoma [1, 9]. However, CGH analyses on benign bone tumors have, to the best of our knowledge, not yet been reported. Analyses of chromosomal instabilities in benign bone tumors may be important to understanding the mechanisms of tumorigenesis by comparing genetic aberrations with those of malignant bone tumors. In this study, we analyzed chromosomal instabilities in 12 benign bone tumors.

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Material and methods

Patients. Twelve benign bone tumors or tumor-like lesions from 12 patients were available for this study (Tab. 1). All samples were obtained from frozen tissues, which were taken and preserved at –80 °C. Tumor tissues were taken from typical and viable tumor areas. In osteoid osteoma, the tissue was obtained from the nidus. In solitary bone cysts and aneurysmal bone cysts (ABCs), the tissues obtained by curettage from the cyst wall were used. Eleven tissues were taken from the primary lesions and one (Case 1) was from a local recurrence. Case 1 underwent curettage; however, local relapse developed 6 months after the initial surgery. The diagnosis was histologically confirmed as a benign bone lesion.

Of 12 lesions, 3 were cartilaginous tumors (including of 2 enchondromas and one chondromyxoid fibroma) and one was an osseous tumor (osteoid osteoma). Eight tumor-like lesions comprised 4 ABCs, one solitary bone cyst, one eo-

Table 1. Patients with benign tumor and results of chromosomal aberrations

Case No.	Age (years)	Gender	Diagnosis	Site	Chr1 p	Chr1 q	Chr12 p	Chr12 q	Chr13 p	Chr13 q	Chr16 p	Chr16 q	Chr17 p	Chr17 q	Chr19 p	Chr19 q	Chr20 p	Chr20 q	Chr22 p	Chr22 q
1	36	Male	Chondromyxoid fibroma	Metatarsal II	L*			L*		G*	L		L		L	L		L		L
2	27	Male	Enchondroma	Metacarpal V	L*					G*	L	L	L	L	L	L	L			
3	43	Female	Enchondroma	Humerus, proximal												L	L			L
4	33	Male	Osteoid osteoma	Femur, diaphysis																
5	13	Female	Aneurysmal bone cyst	Femur, proximal									L		L	L				L
6	14	Female	Aneurysmal bone cyst	Humerus, distal																
7	11	Male	Aneurysmal bone cyst	Calcaneus																
8	18	Male	Aneurysmal bone cyst	Humerus, proximal																
9	9	Male	Solitary bone cyst	Humerus, diaphysis																
10	14	Male	Fibrous dysplasia	Femur, proximal											L	L				
11	11	Male	Eosinophiles granuloma	Femur, diaphysis				G*							L	L				
12	47	Female	Rosai-Dorfmann disease	Radius, diaphysis																

G – chromosomal gain, L – chromosomal loss; *aberrations of small segmented of chromosome.

sinophilic granuloma, and one fibrous dysplasia, and one sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) [14] (Tab. 1). One of 2 enchondoromas abounds of cartilaginous cells and one of 4 ABCs was characterized by fast progression. No case showed local relapse after intralesional (curettage) procedures, excluding previous treatment in Case 1.

Labeling procedures, comparative genomic hybridization and detection. Reference DNA from healthy blood donors and tumor DNA were labeled by the nick translation method with digoxygenin-11-dUTP (Boehringer Mannheim, Germany) and biotin-14-dATP (Boehringer Mannheim, Germany), respectively. The hybridization was performed as described by KALLIONIEMI et al [7] with some modifications [2, 15].

Separate digitized gray level images of DAPI, FITC, and rhodamide fluorescence were taken with a CCD camera connected to a Leica DMRBE microscope. The image processing was carried out using Applied Imaging Software (Cytovision 3.1). Ratio profiles were averaged from 10 metaphases per sample (up to 20 chromosome homologues). Gains of DNA sequences were defined as chromosomal regions with a fluorescence ratio above 1.25 and losses as regions with a ratio below 0.75. A positive control with known aberrations and a negative control were included in each CGH experiment as quality controls. Over-representations were considered to be high-level gains when the fluorescence ratio exceeded 1.5. Heterochromatic regions near the centromeres and the entire X and Y chromosomes were excluded from the analysis. Judgment was based on a consensus of at least two of 3 authors in all cases without reference to the patient's clinical information.

Results

Genomic imbalances were detected in 6 of 12 lesions (Tab. 1). Losses (19 incidences) were more frequent than gains (3 incidences). There were losses of whole chromosome-19's in 6 cases, a loss of chromosome-arm 22q in 4 cases, a loss of chromosome-arm 17p in 3 cases, a loss of chromosome-arm 16p in 2 cases, a loss of chromosome-arm 1p in 2 cases, and a loss of chromosome-arm 16q, 17q, or 20q in one case each. Loss of 1p was limited to the short region of 1p36 and loss of 12 was limited to the region of 12q24. Gains occurred at chromosome 13q21 in 2 cases and at 12q15-q21 in one case.

There were 16 aberrations in 3 cartilaginous tumors, none in one osseous tumor, and 6 in 8 tumor-like conditions. Of the cartilaginous tumors, all showed losses of chromosome 19 and 22q. Case 3 was characterized by a low cellularity and had a less aberrations than either Case 1 or 2; Case 1 was a relapsed tumor and Case 2 was an enchondroma with many cartilaginous cells. One of 4 ABCs had aberrations; this tumor had exhibited rapid growth.

Discussion

To our knowledge, there have been no reports of CGH in these benign bone tumors or tumor-like conditions. An average of 5.3 and 0.75 aberrations were detected in cartilaginous tumors and tumor-like conditions, respectively, with genetic losses being more frequent than gains. Malignant bone tumors have a lot of aberrations with gain dominant. Osteosarcomas have been reported to have an average of 9.6 aberrations [21] or a median of 16.1 aberrations [11]. Ewing tumors [10] and chondrosarcomas [9] exhibit averages of 3.6 and 4.8 aberrations per tumor, respectively.

Solitary enchondroma is a benign cartilaginous tumor mainly arising in the small bones of the fingers with infrequent recurrence. Chondromyxoid fibroma is a benign tumor of bone characterized by a chondroid and myxoid differentiation in its basic tissue, and the chondroid compo-

nent (including pleomorphic tumor cells) can be mistaken for a chondrosarcoma [6]. These lesions have been reported to have a 25% local-recurrence rate [23]. GUNAWAN et al [5] reported a solitary enchondroma with t(8;17)(q23;p13) and loss of chromosomes 9, 19, and 22 as clonal changes. TARKKANEN et al [20] reported a chondromyxoid fibroma with clonal aberrations in chromosomes 2 and 5. In the moderately cellular cartilaginous component of dedifferentiated chondrosarcoma, CGH revealed deletions of chromosomes 4, 5, 13, 22, distal part of 16p, or 17p [1]. Osteoid osteoma is a benign osteoblastic lesion with a limited growth potential; the current case had no detectable chromosomal aberration. DAL CIN et al [3] reported that one of 6 cases of osteoid osteoma showed clonal structural change including chromosomes 1, 6, 14, and 17.

ABCs are benign, but often rapidly expanding, osteolytic lesions [16]. In this study, losses of 17p, 19, or 22q were detected by CGH. In a previous study, a normal karyotype was observed for 8 patients with ABCs [13]. PANOUTSAKOPOULOS et al [12] reported that 2 of 3 ABCs had a t(16;17)(q22;p13) rearrangement and one ABC had a del(16)(q22). SCIOT et al [16] reported that all ABCs showed a non-random involvement of chromosome segments 17p11-13 and/or 16q22.

A loss of whole chromosome-19 was the most frequent abnormality identified in this study. Our observations may indicate the existence of an important gene located on chromosome 19. The information on aberrations of chromosome 19q in human tumors is limited; however, a loss of 19q is commonly observed in gliomas [8, 17, 18].

In summary, a loss of chromosomes was more frequent than a gain of chromosomes in benign cartilaginous tumors. The significance of each individual chromosomal aberration, especially of chromosome-19 loss, will be understood in greater detail with further trials which include larger numbers of benign tumors.

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