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Comparative genomic hybridization of primary skeletal Ewing's sarcoma

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We used comparative genomic hybridization (CGH) technique to identify the genetic changes in 8 primary skeletal Ewing's sarcoma samples from archieved paraffin-embedded tissues from children (4-17 years old) using double step degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR). CGH analysis showed that the most common genetic alterations were losses of chromosomes 1p, 2q, 3p, 14q, 21, X and gains of chromosomes 8q and 17q. Chromosomal deletions were found only in samples taken from males, while amplifications were identified in both sexes. As a result, this difference may explain why tumor occurs about 1.5 times more commonly in males than in females. [Turk J Cancer 2001;31(1):21-26]

Key words: Comparative genomic hybridization, Ewing's sarcoma, DNA amplification and deletion

The benign and malignant bone tumors are relatively rare forms of cancer. Primary cancers of bone are uncommon malignancies in children. Ewing's and osteogenic sarcomas are the two most common forms of childhood bone cancer (1). Ewing's sarcoma is the second most common malignant tumor of bone seen in adolescence and childhood (2). The tumor occurs about 1.5 times more commonly in males than in females (1). There are certain histologic similarities between Ewing's sarcoma and other neuroectodermal originated tumors, and therefore diagnosis is difficult (3). Losses and gains of genetic material which serve as a sign of oncogenes and tumor suppressor genes play an important role in transformation and progression of tumors. Conventional cytogenetic analysis of solid tumors is very essential, but it is too difficult and requires a large number of viable cells. Therefore, complete genetic description that is considered to be important diagnostic criteria is limited in tumors. Cytogenetic analyses have recently revealed a consistent chromosomal translocation t(11;22) (q24;q12) in cell lines derived from Ewing's sarcoma (4). Also, this translocation was found in two extraskeletal Ewing's sarcoma

specimens (5). Recurrent gains of 1q, 8, and 12 in the Ewing family of tumors were found by comparative genomic hybridization (CGH) study according to our knowledge (6). The aim of this study is to describe whether the secondary gains and losses of DNA sequence occur in primary skeletal Ewing's sarcoma samples by CGH (7-10) using double step degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) (11-15).

Materials and Methods

Eight archival formalin-fixed, paraffin-embedded primary skeletal Ewing's sarcoma samples from children (age range between 4-17 years) were obtained from Pathology Department, University of Çukurova, in Turkey. Sections (5 μm) were cut from tumor blocks and stained with hematoxylin and eosin to ensure the histological representation of the sample and cross detecting of areas of tumor cells. Based on microscopic evaluation, areas of interest were identified, scraped with a scalpel, collected in sterile eppendorf tubes containing 50 μl DOP-PCR reaction solution in order to amplify the DNA directly.

CGH experiments and the evaluation of the results were performed as described previously (10-12,15) with minor modifications. Briefly, tumor DNA and normal male or female reference DNA (from normal male and female DNA) were labeled with spectrum green-dUTP and spectrum red-dUTP (Vysis Co.), respectively by applying DOP-PCR.

Metaphase spreads were prepared from phytohemagglutinin stimulated lymphocytes of healthy male (46,XY) and female (46,XX) by standard procedures of colcemide arrest, hypotonic treatment and 3:1 methanol/glacial acetic acid fixation.

For CGH, 500 ng of tumor DNA, 500 ng of normal DNA and 50 μg unlabeled Cot-1 DNA were co-precipitated and redissolved in 15 μl hybridization buffer. DNA and metaphase spreads were denatured at 70°C and at 68°C, respectively.

Hybridization was allowed to proceed for 2 days. Post-hybridization washes were carried out to a stringency of 50% formamide/2xSSC at 45°C. Imaging processing was carried out using Mac Probe Program version 3.4 software. Average green:red fluorescence intensity ratio profiles were calculated for each chromosome in 10 metaphases. Defining the gain and loss in DNA sequence copy number in tumors were based on comparison of normal DNAs labeled with two different colors according to previously described protocol (16,17). The decision limits of the green-to-red ratios were <0.85 for the loss of DNA copy number and >1.40 for the gain of DNA copy number. High level increases were distinguished from low level increase by a cut-off level of 2.0. Also, for DOP-PCR and CGH, various negative and positive control experiments were carried out by crossing different labeled test and control DNAs as well as hybridization on chromosomes.

Results

Comparative genomic hybridization analysis of 8 Ewing's sarcoma samples showed different chromosomal rearrangements. Figure 1 shows chromosomal losses and gains detected by CGH.

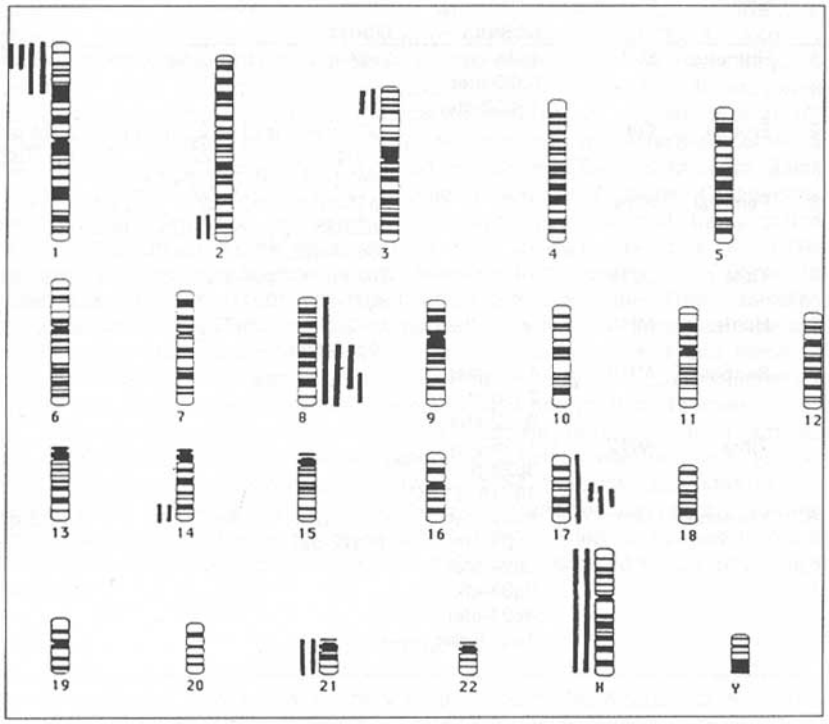

Fig 1. Summary of minimal common regions of all gains and losses found by CGH in 8 skeletal Ewing's sarcoma samples. Vertical lines on the right side of a chromosome, gains of genetic material; vertical lines on the left side, losses

Common losses were observed on chromosomes 1p, 2q, 3p, 14q, 21 and X. However, gains were found on chromosomes 8q and 17q. Of 8 cases, 6 and 2 cases were males and females, respectively. Male:female ratio was 3:1. Chromosomal deletions were only on male chromosomes, while amplifications were identified on chromosomes of both sexes. Four samples (no. 2, 3, 4, 5) showed only amplified sites, in contrast, two patients (no. 6, 7) showed only deleted sites (Table 1). Table 2 presents the most frequent losses and gains detected by CGH in 8 Ewing's sarcomas. Chromosome 1p35-pter deletion was the most frequent deletion. Gains in 8q22-q23 and 17q22 were detected as the most frequent amplifications (Table 2). Other amplifications and deletions detected are presented for each case in Tables 1 and 2.

Table 1
The losses and gains of DNA sequences detected by CGH in 8 skeletal Ewing's sarcoma samples

No	Tumor Site	Sex/Age	DNA sequence copy number change regions	
			Losses	Gains
1	Humerus	M/7	1p34-pter 6q25-qter 13q33-qter	2q24-q32, 7p21-p22, 8q13-qter, 17q23-25
2	Frontal	F/4		1p21-pter, 3p21-p22, 4p14-p16, 5p14-p15, 8, 9q31-q34, 13q22-q32, 15q21, 17q22, 18q22-q23, 20, 22q12-q13
3	Femoral	F/14		2p15-pter, 2q21-q24, 3q23-q25, 4, 5, 6p23-25, 7, 8q22-q24, 9p22-pter, 12, 15q23-q26, 17, 18, 20q13-qter
4	Tibia	M/17		2p23-pter, 3p24-pter, 4q27-qter, 9p21-p23, 9q31-qter, 10, 11q, 15, 17q11-q22, 18p
5	Humerus	M/16		1q42-qter, 3p22-p23, 3q27-qter, 6, 19p13-pter
6	Scapula	M/16	1p35-pter 2q36-qter 6p22-pter	
7	Tibia	M/12	1p35-pter 3p23-pter 14, 18, 21, X	
8	Humerus	M/9	1p34-pter 2q36-qter 3p24-pter 5q33-qter 8p21-pter 14q31-qter 21,X	5q14-q23, 8q21-q23, 12q13-q15, 17q12-q22

Table 2
The most frequent losses and gains detected by CGH in 8 skeletal Ewing's sarcoma samples

Chromosome	Minimal common region	Sex (M/F)	Frequency in 8 samples
Losses			
1p	1p35-pter	4/-	4
2q	2q36-qter	2/-	2
3p	3p24-pter	2/-	2
14q	14q31-qter	2/-	2
21	21	2/-	2
X	X	2/-	2
Gains			
8q	8q22-23	2/2	4
17q	17q22	2/2	4

Discussion

We used CGH technique, to investigate chromosomal abnormalities that have been considered to be involved in transformation and progression of the Ewing's sarcomas. We found different losses and gains (Table 1). Although few samples were tested, results obtained were suggested to be important in Ewing's sarcoma. Deletions were found on chromosomes 1p, 2q, 3p, 14q, 21 and X. Chromosome 1p35-pter deletion was detected as the most frequent deletion. Also, chromosome 1p deletion is a common chromosomal abnormality in tumors and harbour some oncogenes (18). Argenmol et al. (6) found recurrent gains of 1q, 8 and 12 chromosomes in Ewing's sarcoma by CGH. In our study, although we have had few cases of Ewing's sarcoma, gains of the chromosome 8q22-23 were found in four of 8 cases. Cytogenetic analysis have recently revealed a consistent chromosomal translocation t(11;22) (q24;q12) in cell lines derived from Ewing's sarcomas (4). CGH technique is not able to identify any balanced translocation. CGH allows to identify amplifications and deletions in whole tumor genome. These samples may have this specific translocation, as well as additional amplifications and deletions on other chromosomes, too. This is because losses and gains of genetic material which serve as a sign of oncogenes and tumor suppressor genes play an important role in transformation and progression of tumors.

The tumor occurs about 1.5 times more commonly in males than in females (1). Although we have few samples randomly selected, male:female ratio was 3:1. Chromosomal deletions were only found on male chromosomes in this study (Table 1). These results may explain why tumor occurs about 1.5 times more commonly in males than in females. If data would be increased, more informative results should be obtained to define the restricted site(s) for Ewing's sarcoma.

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