Molecular cytogenetic analysis of oral squamous cell carcinomas by comparative genomic hybridization, spectral karyotyping, and fluorescence *in situ* hybridization

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Abstract

We investigated relationships between DNA copy number aberrations and chromosomal structural rearrangements in 11 different cell lines derived from oral squamous cell carcinoma (OSCC) by methods of comparative genomic hybridization (CGH), spectral karyotyping (SKY), and fluorescence *in situ* hybridization (FISH). CGH frequently showed recurrent chromosomal gains of 5p, 20q12, 8q23-qter, 20p11-p12, 7p15, 11p13-p14, and 14q21 and losses of 4q, 18q, 4p11-p15, 19p13, 8p21-pter, and 16p11-p12. SKY identified repetitive translocational chromosomes: i(5)(p10), i(5)(q10), i(8)(q10), der(X;1)(q10;p10), der(3;5)(p10;p10), and der(3;18)(q10;p10). In addition, breakpoints detected by SKY were clustered in 11q13 and around centromeric regions, including 5p10/q10, 3p10/q10, 8p10/q10 14q10, 1p10/1q10, and 16p10/16q10. Cell lines with i(5)(p10) and i(8)(q10) showed gains of entire chromosome arm of 5p and 8q by CGH, respsctively. Moreover, breakages near the centromeres of chromosomes 5 and 8 may be associated with 5p gain, 8q gain, and 8p loss in OSCC. FISH with a DNA probe from a BAC clone mapping to 5p15 showed a significant correlation between the average numbers of i(5)(p10) and 5p15 (R^2=0.8693, P<0.01) in these cell lines, indicating that DNA copy number of 5p depends upon

isochromosome formation in OSCC.

1. Introduction

Oral cancer, predominantly oral squamous cell carcinoma (OSCC), is one of the most common cancers worldwide [1,2]. In some countries in Southeast Asia, OSCCs constitute approximately one-third of all cancers [3]. Despite recent progress in diagnostic and therapeutic modalities for OSCC, the prognosis has not improved since the 1960s and is particularly poor for patients with advanced-stage OSCC [4]. Biologic characteristics of tumors are primarily affected by genetic changes in tumor cells. Information regarding biologic characteristics of cancer cells may be useful in improving treatment and evaluating patient prognosis.

It is widely accepted that various carcinomas, including OSCC, result from the accumulation of genetic alterations within a cell [5, 6]. Numeric and structural changes of chromosomes play important roles in the accumulation of genomic aberrations that lead to oncogenesis [7]. Conventional cytogenetic studies of head and neck squamous cell carcinoma (HNSCC) have revealed recurrent chromosomal aberrations including i(8)(q10), i(5)(p10), i(1)(q10), del(3)(p11p12), del(5)(p11), t(1;1)(p13;q25), and der(14;15)(q10;q10). In addition, it has been reported that chromosomal bands 11q13 and 1p13 and those near centromeres in chromosomes 1, 3, 5, 7, 8, 13, 14, and 15 are frequently involved in HNSCC [8]. However, it can be difficult to prepare metaphase spreads of primary HNSCCs as well as other solid tumors. Therefore, karyotypic analysis is possible in limited cases. Comparative genomic hybridization (CGH) is a powerful tool that allows global analysis of DNA copy number changes across the entire genome in a single experiment [9]. We have analyzed HNSCCs, including OSCCs [10-12], by CGH and identified recurrent DNA copy number aberrations, such as gains of chromosomes 3q, 5p, 8q, 12p, 20, and X and deletions of chromosomes 3p, 4, 8p, 13q, 17p, and 18q. Karyotyping or CGH analysis has detected frequent aberrations of chromosome 5 in OSCC cells. However, there are no reports concerning the relation between structural abnormalities and DNA copy number aberrations of chromosome 5 in OSCC.

Spectral karyotyping (SKY) allows instantaneous visualization of defined emission spectra for each human chromosome after fluorescence *in situ* hybridization (FISH) and helps detailed analysis of complicated structural abnormalities of chromosomes [13]. Combined CGH and SKY have been performed to some solid tumors, including cervical cancer [14], esophageal cancer [15], and colon cancer [16].

In HNSCCs, there are a few reports using methods of CGH and SKY [17,18], furthermore, there are no reports focused on OSCC.

In this study, we applied CGH, SKY and FISH to 11 human OSCC cell lines to investigate the relation between DNA sequence copy number aberrations and structural rearrangements of chromosomes.

2. Materials and Methods

2.1. Cell lines

Eleven human OSCC cell lines derived from Japanese patients (KM-2, Sa-3, H-1, BHY, HN, TYS, OSC30, OSC70, SAS, HSC-3, and HSC-4) were used in the present study. Clinical characteristics of the cell lines, including patient age, sex, site, and histological differentiation, are summarized in Table 1. KM-2 was established at our laboratory, and Sa-3, H-1, BHY, HN, TYS, OSC30, and OSC70 were gifts from the listed institutions (Table 1). SAS was obtained from the Japanese Collection of

Research Bioresources Cell Bank (Tokyo, Japan), and HSC-3 and HSC-4 were purchased from the Health Science Research Resources Bank (Osaka, Japan). All cell lines were grown in Dulbecco’s modified Eagle medium (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (Equitech-Bio, Ingram, TX). All cultures were harvested at the proliferating phase. Colcemid (0.025 μ g/ml) was added 90 min before harvesting, and cells were harvested by trypsinization. After hypotonic shock with 0.056 M KCl/0.7 mM trisodium citrate dihydrate solution, cells were fixed three times in methanol-acetic acid (3:1) and dropped onto slides. Chromosome spreads were prepared with a HANABI® metaphase spreader (ADScience Technologies Co., Ltd., Chiba, Japan).

2.2. CGH

Test DNA was isolated from the cell lines, and reference DNA was isolated from peripheral lymphocytes of healthy volunteers with a DNA extraction kit (SepaGene; Sankojyunyaku Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions. CGH was performed as described previously [19]. In brief, DNA extracted from cell lines was labeled with SpectrumGreen-dUTP (Vysis, Inc., Downers Grove, IL) by nick translation, and sex-matched reference DNA was labeled with SpectrumRed-dUTP (Vysis, Inc.). These labeled DNAs (200 ng each) were mixed with 10 μg Cot-1 DNA in 10 μl hybridization buffer (70% formamide, 10% dextran sulphate, and 2 × SSC, pH 7.0) and applied to normal denatured metaphase chromosome spreads. At least 10 metaphase spreads were digitized and analyzed with a QUIPS XL genetics workstation system (Vysis, Inc.). Increases (gains) and decreases (losses) in DNA sequence copy number were defined by tumor/reference ratios >1.2 and <0.8, respectively. High-level gains of chromosomal regions were defined by tumor/reference ratios >1.5.

2.3. SKY

Metaphase slides were denatured and hybridized with a SKY Paint Kit® (Applied Spectral Imaging, Ltd., [ASI], Migdal Haemek, Israel) according to the manufacturer’s protocol. After incubation at 37˚C for 48 h, metaphase slides were washed and counterstained with 4’,6-diamidino-2-phenylindole in the antifade solution supplied with the kit. Images of cells were obtained with a CCD camera (Spectra Cube®, ASI), and 10 metaphase cells were studied for each sample. Images were analyzed by SKY View® (ASI). Numeric or structural aberrations detected in more than four metaphase cells (>40%) were considered clonal changes.

2.4. FISH

FISH was performed as reported previously, with a minor modification [20]. In brief, a BAC clone (RP11-43F13 on 5p15) was amplified with DOP-PCR and labeled by nick translation with SpectrumGreen-dUTP [21]. Specificity of the probe was confirmed by FISH-mapping onto normal lymphocyte metaphases. For each cell line, 25 metaphases were analyzed, and the numbers of 5p15 FISH signals and isochromosomes of 5p were counted.

2.5. Statistical Analysis

Values are expressed ±standard deviation. Statistical analysis was performed with JMP 4.0® software (SAS Institute, Inc., Cary, NC). Pearson's correlation coefficient was used to determine the association between the numbers of i(5)(p10) and 5p15 signals. For all statistical tests, $P<0.05$ was considered significant.

3. Results

3.1. Overall genome copy number aberrations based on CGH

Genetic alterations detected by CGH are summarized in Fig. 1. The total number of genetic aberrations ranged from 15 to 31 (23.4±4.1 per cell line). The average number of DNA sequence copy number gains, losses, and high-level gains was 12.1±2.5, 11.3±3.5, and 3.0±1.5, respectively. Gains were detected frequently at chromosomal regions of 5p (100% of 11 cell lines), 20q12 (91%), 8q23-qter (82%), 20p11-p12 (73%), 7p15 (64%), 11p13-p14 (64%), 14q21 (64%), 3q26-qter (55%), 9q34 (55%), and 11q13 (45%), and losses were detected frequently at 4q (100%), 18q (91%), 4p11-p15 (73%), 19p13 (73%), 8p21-pter (64%), 16p11-p12 (64%), 3p23-ter (55%), 10p13-pter (55%), 21q22-qter (55%), 7q33-qter (45%), and 18p (45%). Repetitive high-level gains were found at 5p15 (73%), 8q24 (45%), 20p11-p12 (45%), 20q13 (45%), 7p (18%), and 14q22-24 (18%).

3.2. Karyotyping analysis based on SKY

SKY data for the 11 cell lines are summarized in Table 2, and SKY images of cell line SAS are shown in Fig. 2. A total of 215 kinds of derivative chromosomes were detected by SKY (mean 21.7±7.1 per cell line): 244 translocations, 19 deletions, 5 insertions, 7 duplications, 10 homogeneously staining regions, and 8 fissions. 57 of these derivative chromosomes contained 2 or more structural chromosome aberrations. Robertsonian translocation, der(14;15)(q10;q10), was observed in 2 cell lines (Sa-3 and H-1). Four kinds of isochromosomes were identified recurrently, including i(5)(p10) in 8 cell lines (KM-2, Sa-3, H-1, BHY, HN, TYS, OSC70, and SAS), i(5)(q10) in 4 cell lines (TYS, OSC30, SAS, and HSC-4), i(8)(q10) in 4 cell lines (TYS, OSC30, OSC70, and SAS), i(7)(p10) in 2 cell lines (Sa-3 and TYS), and i(16)(q10) in 2 cell lines (OSC30 and TYS). Translocations involving entire chromosome arms were also noted, including der(X;1)(q10;p10) in 4 cell lines (Sa-3, BHY, OSC30, and HSC-3), der(3;5)(p10;p10) in 3 cell lines (BHY, OSC30, and HSC-3), and der(3;18)(q10;p10) in 3 cell lines (Sa-3, TYS, and HSC-3). No repetitive reciprocal translocations were observed.

The distribution of chromosome breakpoints is illustrated in Fig. 1. A total of 468 breakpoints were observed in the 11 cell lines (mean 42.5±17.7 per cell line), and 315 of these breakpoints were identified in rearranged chromosomes. 94 of 315 identified breakpoints were located in or near centromeric regions, and others were mapped to chromosomal bands or terminal regions. An additional 152 breakpoints were unidentified. Breakpoints were frequently found in 11q13 (64% of 11 cell lines) and near centromeric regions, such as 5p10/q10 (100%), 3p10/q10 (82%), 8p10/q10 (73%), 14q10 (64%), 1p10/1q10 (55%), 16p10/16q10 (55%), Xp10/Xq10 (45%), and 11p10/11q10 (45%).

3.3. Relations between recurrent chromosomal structural rearrangements and DNA copy number aberrations

Relations between recurrent chromosomal rearrangements and DNA copy number aberrations are summarized in Table 3. All cell lines with i(5)(p10) and i(8)(q10) showed a gain of 5p and 8q respectively, by CGH. In addition, 2 cell lines with i(5)(q10) showed a gain of 5q by CGH. Gains of 5p and 8q and loss of 8p were detected frequently by CGH, whereas gain of 5q was detected in only 3 cell lines.

All 3 cell lines with der(3;5)(p10;p10) showed a loss of 3p and a gain of 5p by CGH. Two of 4 cell lines with der(X;1)(q10;p10) exhibited a gain of Xq. One of 3 cell lines with der(3;18)(q10;p10) showed a gain of 3q and a loss of 18p.

3.4. FISH analysis of 5p

To obtain more information regarding 5p aberrations, we performed FISH analysis with a probe from BAC clone RP11-43F13 (Fig. 2D, arrows). The average number of RP11-43F13 signals per cell line ranged from 3.08 to 9.40 (Table 4). Cell lines with i(5)(p10) showed more signals than those lacking it. There was a significant correlation between the average number of i(5)(p10) and the number of RP11-43F13 signals ($R^2=0.8693$; $P=0.0001$). This relation was proved with a regression line, $y=1.51x+3.35$ (Fig. 3), where y is the average number of RP11-43F13 signals, and x is the average number of i(5)(p10).

4. Discussion

In this study, comprehensive molecular cytogenetic analyses of 11 OSCC cell lines were performed with CGH, SKY, and FISH. CGH analysis revealed that genomic aberrations in OSCC cell lines used are quite similar. However, SKY analysis revealed complex and unique karyotyping patterns in each cell line (Fig. 1 and Table 2). This discrepancy may be due to characteristics of the carcinomas studied. Similar results have been also observed in other solid tumors analyzed by CGH and SKY [14-18].

A total of 94 chromosomal breakpoints were detected around centromeric regions, and they were frequently found in chromosomes 1, 3, 5, 8, 11, 14, 16, and X (Fig. 1). Centromeric chromosomal breakages are a characteristic cytogenetic feature of squamous cell carcinomas, including OSCCs [17,22,23]. The present study also showed frequent centromeric chromosomal translocations identified as isochromosomes, whole-arm translocations, or Robertsonian chromosomes. Most were associated with hot spots of centromeric chromosomal breakpoints (Table 3). Recent studies with traditional G-band techniques showed that i(5)(p10) and i(8)(q10) are recurrent derivative chromosomes in HNSCC [8]. We detected i(5)(p10) in 8 cell lines and i(8)(q10) in 4 cell lines. Furthermore, we identified i(5)(q10), der(X;1)(q10;p10), der(3;5)(p10;p10), and der(3;18)(q10;p10) in more than 2 OSCC cell lines. Because many derivative chromosomes were unique, recurrent derivative chromosomes may indicate characteristic chromosomal aberrations in OSCC.

In our study, cell lines with i(5)(p10) and i(8)(q10) always showed gains of 5p and 8q, respectively, whereas other isochromosomes were not always associated with chromosomal gains of the corresponding region by CGH. This indicates that i(5)(p10) and i(8)(q10) are particularly important chromosomal aberrations resulting in increased copy number of chromosomes 5p and 8q in OSCC. These aberrations are associated with OSCC, but it remains unknown whether they cause or are a consequence of OSCC.

Chromosomes 5 and 8 were frequently increased in number (Table 3) However, CGH analysis of the 11 cell lines detected frequent gains of 5p and 8q and losses of 8p (Fig. 1). These findings suggest that particular centromeric breakpoints near the centromeres of chromosomes 5 and 8 are associated with 5p gain, 8q gain, and 8p loss in OSCC. Accordingly, these changes are detected as isochromosomes.

We observed several consistent changes in this study, indicating gain of 5p and loss of 4q by CGH and breakpoints of 5p10/q10 by SKY. These cahanges may be fundamental aberrations of OSCC. Reported candidate genes located in 4q and 5p are REST (4q13) and SKP2 (5p13), h-TERT (5p15), and TRIO (5p15) [24-27].

FISH with DNA probe RP11-43F13 mapping to 5p15 revealed a significant correlation between increased numbers of 5p15 and i(5)(p10) (R^2=0.8693; P=0.0001; Fig. 3). This result suggests that DNA copy number of 5p depends upon isochromosome formation in OSCC. In addition, we previously reported that a gain of 5p was associated with high pathologic stage, nodal metastasis, and poor prognosis in esophageal squamous cell carcinoma [28]. In HNSCCs, a gain of 5p14-pter has been reported to be associated with short-term survival after surgery [29]. Increased 5p15 signal number and appearance of i(5)(p10) may constitute markers for estimating disease progression in OSCC. Large-scale studies of clinical samples will be necessary to examine this possibility.

In conclusion, we report cytogenetic properties and relations between recurrent numeric genomic alterations and derivative chromosomes in 11 OSCC cell lines by CGH, SKY, and FISH. Among these chromosomal aberrations, i(5)(p10) was closely associated with a gain of 5p. Although our findings are the results from cell lines, we believe that the resent results are useful to design a basic or clinical research of OSCC.

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Figure Legends

Fig. 1. Summary of DNA sequence copy number aberrations detected by CGH and of chromosomal breakpoints identified by SKY in 11 OSCC cell lines. CGH results are shown in the left panel of each chromosome ideogram. Vertical lines to the left of each chromosome ideogram represent losses, and those to the right represent gains. Dotted lines to the right of each chromosome ideogram show high-level gains. Breakpoints identified by SKY are marked in the right panel of each chromosome ideogram. Asterisks to the right of each chromosome ideogram represent the number of cell lines showing breaks at that locus.

Fig. 2. (A)-(C) Images of SKY analysis from a representative metaphase spread of cell line SAS. Images show (A) spectral image, (B) inverted DAPI image, and (C) pseudocolor image. (D) FISH with RP11-43F13 in metaphase spreads of cell line SAS shows i(5)(p10) as small metacentric chromosomes with signals in both terminal regions (arrows).

Fig. 3. Correlation analysis of the relation between the average numbers of i(5)(p10) and 5p15 signals. A significant correlation was identified (R^2=0.8693; *P*=0.0001). Regression line: y=1.51x+3.35.

Table 1

The 11 oral squamous cell carcinoma cell lines and source information

Cell line	Age (yr)/Sex	Tumor site	Differentiation	Institute
KM-2	60/M	Tongue	Well	Yamaguchi
Sa-3	63/M	Gingiva	Well	Wakayama
H-1	55/M	Gingiva	Moderate	Wakayama
TYS	80/F	Mouth floor	Well	Tokushima
HN	55/M	Soft palate	Moderate	Tokushima
BHY	52/M	Gingiva	Well	Tokushima
OSC30	78/F	Tongue	Well	Sapporo
OSC70	61/M	Tongue	Well	Sapporo
SAS	69/F	Tongue	Poor	Chiba
HSC-3	63/M	Tongue	Poor	Tokyo Med/Dental
HSC-4	63/M	Tongue	Well	Tokyo Med/Dental

Abbreviations: Differentiation, histological differentiation of squamous cell carcinoma; Institute, Institute where cell line was established; Yamaguchi, Department of Oral and Maxillofacial Surgery, Yamaguchi University; Wakayama, Department of Oral and Maxillofacial Surgery, Wakayama University; Tokushima, Second Department of Oral and Maxillofacial Surgery, Tokushima University; Sapporo, Department of Oral and Maxillofacial Surgery, Sapporo Medical College; Chiba, Department of Oral and Maxillofacial Surgery, Chiba University; Tokyo Med/Dental, First Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tokyo Medical and Dental University.

Table 2

SKY karyotypes of the11 oral squamous cell carcinoma cell lines

Cell line	Karyotypes
KM-2	49-53, X, der(Y)(Y;19)(q10;?), der(Y)(Y;12)(q10;q10), der(1;14)(p10;q10), +der(2)add(2;5)(qter;?), der(4)t(4;13)(q31;q?), der(4)t(4;9)(q32;?), +i(5)(p10), der(5)add(5;15)(qter;q?), +der(5)t(3;5)(?;?)t(5;3;4)(?;?;?), der(6)t(6;15)(p21;q?)dup(6)(q22q23)add(1;6)(?;qter), der(6)t(6;13)(p12;q?), der(7)t(6;7)(p21;q32), der(8)t(1;8)t(?;p21), der(8)t(8;11)(p22;?), +der(8)t(8;11;19)(p23;?;?)ins(8;Y)(q?;q?), der(10)t(7;10)(?;p15), der(12)t(4;12)(q31;q24), der(13)t(5;13)(q21;p12), +14, der(14)t(14;19)(q10;?)dup(19)(?), +i(16)(p10), der(16)t(15;16)(q?;q24), der(16)t(10;16)(?;q22), der(17)t(17;17)(q25;q25q21)add(17;19)(q21;?), der(17)t(12;17)(?;q21), -18, der(18)t(12;18)(?;q21), der(19)t(9;19)(?;?), der(19)t(9;19)(q21;?)t(19;22;1;6)(?;q?;?;?), hsr(20)(p11p12), der(20)t(8;20)(?;?), der(20;21)(?;q10), -21
Sa-3	98-100, X, der(X;1)(q10;p10), der(X)t(X;9)(q13;?), Y, der(1)t(1;3)(p22;q12), der(1;11)(q10;q10), der(1;11)(p10:q10), der(1;13)(q10;q10)t(1;2)(q32;?), der(1;15)(q10;q10), der(2)t(2;5)(p21;?), der(3;18)(q10;p10), der(3;11)(p10;p10), +der(3)t(3;5)(?;q)del(3)(?), der(5;10)(p10;q10),+ i(5)(p10)×3, der(6)t(6;10)(?;q21)t(6;3)(?;p11), +6, +7, i(7)(p10), der(7)t(4;7)(?;q2), der(7)t(7;15)(p15;q?), +fis(7)(p10), der(8)t(8;19)(q10;q10), +del(8)(q22), -9, der(9)t(9;13)(p13;q?), -10, +fis(11)(p10)×2, der(12)t(1;12)(q32;q14), der(13)t(12;13)(q14;q32), der(14;15)(q10;q10), der(16)t(5;16)(?;p13), der(18)t(4;18)(q11;q11), -19, +20 ×2, der(22)t(12;22)(?;p?)
SAS	60-69, X, X, der(X;3)(p10;q10)×2, der(1)t(1;12)(p36;q13), +der(3)add(3;22)(pter;q?), -4, +i(5)(p10)×2, +5, i(5)(q10), -6, +der(7)t(7;11)(q22;q11)del(11)(q22), i(8)(q10), -10, -11, der(11)add(11;11)(qter;q13q13), -12, +fis(12)(p10p11::hsr::p13)×3, +13, der(16)t(16;12;3)(p12;?;?), der(16)t(10;16)(p11;q24), -18, der(18)t(16;18)(?;p11)ins(8;18)(q22::hsr::q24;q21), -19, +der(20)t(6;20)(q21;p13), -21, -22
H-1	62-65, X, der(X)t(X;9)(q21;q34), der(X)add(X;12)(qter;q13), -Y, der(1;3)(q10;p10), +fis(1)(p10), der(1)t(1;3)(p22;q25), der(2)t(2;16)(q24;p11)del(2)(p23), +der(3)t(3;5)(?;q10)del(3)(p?), der(3)del(3)(p?)del(3)(q?), -4, der(4)t(4;20)(p15;p11)add(1;4)(?;qter), der(4;18)(q10;p10)del(4)(q32), i(5)(p10)×3, +5, -6, der(6)add(3;6)(?;qter), der(7)t(4;7)(p15;q22), +der(7)t(7;15)(p22;q?), der(8;16)t(p11;?), -9, der(9)t(9;13)(p11;q31), der(10)t(3;10)(q13;p13), der(10)t(10;12)(q22;?), der(11)t(11;10;22;21)(q13;?;q;q), i(11)(p10)add(9;11)(?;pter)add(11;11)(pter;q13), -12, der(12)t(12;16)(q13;q22), -13, der(14;15)(q10;q10), -16, +fis(16)(p10), i(16)(q10)t(5;16)(q12;q22), -18, -19, -20, -22
TYS	75-78, X, X, X, der(1)add(1;13)(qter;q31), del(2)(q24q32), der(3;18)(q10;p10), -4, der(5;14)(q10;q10)add(5;14)(qter;q11), +i(5)(p10)×4, i(5)(q10), t(5;7)(q31;q32), +fis(6)(p10), +7, i(7)(p10), t(8;10)(p23;p11), +i(8)(q10), +9, -10, +der(11)t(8;11)(?;q13), der(13)t(13;17)(p11;q22), der(14;16)(q10;p10), der(15)t(3;15)(q25;p11), -15, i(16)(q10), -18, der(18)(6;18)(?;q23), +20×3, -21, der(22)t(X;22)(?;q11), +22
BHY <3n>	60-67, X, der(X;1)(q10;p10), Y, -1, der(2)del(2)(q21)add(2;2)(pter;q21), der(2)t(2;10)(q21;q22). +der(3;5)(p10;p10), der(3;17)(q10;q10)×2, der(4)t(1;4)(p13::hsr::p22pter;q21), der(5)t(5;13)(p15;q22)×2, der(5;17)(p10;q10)del(17)(q24), der(5)t(5;14)(q10;p10)del(14)(q24), +i(5)(p10), -6, der(8;10)(q10;q10)×2, der(9)t(9;12)(q21;?), der(10)(7;10)(?;q22), der(10)t(10;12)(?;q21)del(10)(?), +der(11)hsr(11)(q13q13)t(1;11)(q13;q13), der(12)t(12;22)(q21;q?), -13, -14, -16, der(16)dup(16)(q12qter)add(6;16)(p12;qter), -17, -18, der(18)(1;18)(p13;q11), der(19)t(5;19)(q23;p13), +20×2, -21

Table 2

Continued

Cell line	Karyotypes
HN <2n>	49-54, X, der(1)t(1;10)(p32;?)del(1)(q42), der(1)t(1;2)(p32;p14)t(1;14)(q21;q12), der(2)t(2;16)(p13;q21), +der(2)add(2;18)(qter;q21), der(2)t(2;6;16)(p13;?;q21)del(2)(q35), +der(2)t(2;22;1;6)(p14;q?;?;?), der(3)t(3;8)(p21;?), der(4)t(4;9)(q31;?), +5, i(5)(p10), der(6)t(6;13)(p11;q32), der(6)t(6;22)(p11;q11)hsr(6)(q22q23)add(1;6)(?;qter), +der(7)t(6;7)(p11;q31)add(7;12)(qter;?), der(7)t(6;7)(p11;q31), +der(8)t(1;8)t(p32;p21), der(8)t(8;11)(p21;p12)t(3;8)(?;q22), der(8)ins(Y;8)(q?;q?), -9, der(10)t(9;10)(?;?)t(10;17)(?;?), der(10)t(7;10)(q31;p13)t(3;10)(?;q25), +der(12)t(Y;12)(q12q11;q11), der(12;17)(p10;p10), der(13)t(5;13)(q23;p12), der(14)t(14;17;19;17;11)(p11;?;?;?:q), -15, der(15)t(1;15)(?;p11), -16, +fis(16)(q10), der(16)t(15;16)(q?;q22), der(16)t(10;16)(?;p?)t(10;16)(?;q?), der(17)dup(17)(q23q35)add(17;19)(qter;?), der(18)t(3;18)(?;?)t(3;4;18)(?;?;?), der(18)t(12;18)(?;q21), +der(19)t(Y;19)(q12q11;?), der(19)t(9;19)(?;?), der(19)t(2:19)(p14;?)t(9:19)(?;?), +der(19)t(15;19)(q?;?), +20, hsr(20)(p11p12), der(20)t(8;20)(q23;q12), der(20;21)(q10;q10), -21
OSC30 <3n>	66-70, X, der(X)(X;1)(q10;p10), der(1)t(1;8)(p32;q23), +der(2)t(2;8)(q11;q23), +3, der(3)add(3;11)(pter;q13q23), der(3;20)(q10;q10)dup(20)(q12q13)add(9;20)(?;qter), der(3)t(3;7)(q10;p10), der(3;5)(p10;p10), -4, der(5)dup(5)(q31q35)add(5;16)(qter;?), +fis(7)(p10)×2, der(7)t(7;11)(q32;?), i(8)(q10), -9, der(9)t(9;20)(q22;?), der(10)t(10;12)(q24;?), der(11;22)(q10q23;q10)add(4;11)(?;q23)×2, der(11)hsr(q14q23)t(9;11)(q22;qter), der(12)add(1;12)(q11;q24), der(13)add(9;13)(q31;qter)×2, der(14)t(14;21)(q31;q?), i(16)(q10), +17, -18, -21
OSC70 <3n>	64-82, X, X, Y, Y, der(3;21)(q10;q10)×2,+3, der(3)t(3;17)(?:?)del(3)(?), -4, del(4)(q13), +i(5)(p10), der(5)ins(3;5)(?;q?), -7, i(8)(q10)×2, +8, +9, +11, der(14)t(2;14)(p11;q31), der(15;15)(q10q21;q10q21), +der(17)t(17;7;11)(q11;p11::hsr::p15;?), +18, del(18)(q12)×2, +der(20)add(11;20)(?;pter)×2, der(22;22)(q10;q10), der(22)t(22;14)(p13;q21::hsr::q24)t(14;12;19)(q24;?;?)
HSC-3 <3n>	61-63, der(X;1)(q10;p10), der(X;8)(q10;q10), Y, i(1)(q10), der(1;3)(p10p33;q10), del(2)(q21), +fis(2)(p10), der(3;5)(p10;p10), der(3)t(3;11)(p11;q13), der(3;18)(q10;p10), der(3)t(3;22;11;13)(p12;q?;?;q?)dup(3)(q22q26), der(4)t(4;6)(q13;?), der(4)t(4;9)(p13;p21), der(4)t(4;5)(q22;q22), del(6)(q21), -7, +der(9;16)(p10;q10), -10, der(11)t(10;11)(q11;q13), +der(11)t(11;5)(p13;p11)ins(X:11)(p11p22;q13), der(11;22)(p10;q10), der(13;13)(q10;q10), +14, -15, -16 , der(17)t(17;14:8;12)(p11;?;?;?), der(18)t(4;18)(p13;q11), -18, der(19)t(19:11;20)(p13;?;?), -21, der(22)t(22;6;16)(p11;?;p11)t(4;22)(q22;q13), der(22)t(6;22)(p21;q13)
HSC-4 <3n>	78-83, X, X, -Y, +1, t(1;7)(p35;q31)×2, t(1;7)(p35;p15), +2, t(2;7)(q13;q31), der(2)t(2;7)(q13;q31), +3, der(3;8)(q10;q10), -4, i(5)(q10), +der(7)t(7;20)(q11;?), +7, +8, der(8)t(8;13)(p11;q21), +9, der(10;13)(q10;q10), +11, der(11;14)(q10q13::hsr::q13;q10)t(9;11)(?;q13), t(12;17)(q24;q24), der(12;13)(p10;q10q21)add(11;13)(?;q21), der(14)hsr(q21q24)t(14;22)(q24;q13), +14, +15, +20 ×3, der(20)t(9;20)(p10;q10), der(20)t(17;20)(?;p11), -21, -22

Table 3

Summary of recurrent DNA copy number aberrations of chromosomes and chromosomal structural rearrengements

	DSCNAs (frequency)		Karyotypic abnormalities detected by SKY (frequency)		
Chromosome	p arm	q arm	CB	NA	Recurrent derivative chromosomes
X			(5/11)		der(X;1)(q10;p10) (4/11)
1			(6/11)		der(X;1)(q10;p10) (4/11)
2				+ (5/11)	
3	loss (6/11)	gain (6/11)	(9/11)	+ (7/11)	der(3;5)(p10;p10) (3/11), der(3;18)(q10;p10) (3/11)
4	loss (8/11)	loss (11/11)		- (6/11)	
5	gain (11/11)		(11/11)	+ (8/11)	der(3;5)(p10;p10) (3/11), i(5)(p10) (8/11), i(5)(q10) (4/11)
6					
7	gain (7/11)	loss (5/11)		+ (7/11)	i(7)(p10) (2/11), fis(7)(p10) (2/11)
8	loss (7/11)	gain (9/11)	(9/11)	+ (6/11)	i(8)(q10) (4/11)
9		gain (6/11)			
10	loss (6/11)				
11	gain (7/11)	gain (5/11)	(5/11)	+ 6/11	hsr(11)(q13q13) (2/11)
12					
13					
14		gain (6/11)	(7/11)		der(14;15)(q10;q10) (2/11), hsr(14)(q21q24) (2/11)
15					
16	loss (7/11)		(6/11)		i(16)(q10) (2/11)
17					
18	loss (5/11)	loss (10/11)		- (6/11)	der(3;18)(q10;p10) (3/11)
19	loss (7/11)				
20	gain (8/11)	gain (10/11)		+ (7/11)	hsr(20)(p11p12) (2/11)
21		loss (6/11)		- (8/11)	
22					

Abbreviations: DSCNAs, DNA sequence copy number aberrations detected by CGH in more than 4 cell lines; CB, Centromeric breakage detected in more than 4 cell lines; NA, Numerical aberration detected in more than 4 cell lines; +, increase number of the chromosome, -, decrease number of the chromosome.

Table 4

Average numbers of i(5)(p10) and 5p15 detected by FISH with RP11-43F13

Cell line	i(5)(p10) (mean ± SD)	5p15 (mean ± SD)
KM-2	1.00 ± 0	3.92 ± 0.08
Sa-3	2.92 ± 0.40	8.28 ± 0.79
H-1	3.36 ± 0.70	8.00 ± 1.12
HN	1.04 ± 0.20	4.32 ± 1.03
TYS	3.60 ± 0.50	9.40 ± 1.04
BHY	0.80 ± 0.41	5.36 ± 0.76
OSC30	0	3.92 ± 0.28
OSC70	1.76 ± 1.16	6.68 ± 2.17
SAS	1.76 ± 0.44	4.52 ± 0.96
HSC-3	0	3.88 ± 0.44
HSC-4	0	3.08 ± 0.76