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• *GASTRIC CANCER* •

Recurrent chromosomal rearrangements at bands 8q24 and 11q13 in gastric cancer as detected by multicolor spectral karyotyping

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Abstract

AIM: To identify chromosomal translocations specific to gastric cancer (GC), spectral karyotyping (SKY) analysis was performed on established cell lines and cancerous ascitic fluids.

METHODS: SKY analysis of 10 established cell lines and seven cancerous ascitic fluid samples identified recurrent chromosomal breakpoints and translocations in GC, several of which involved chromosomal loci of oncogenes or tumor suppressor genes.

RESULTS: A total of 630 chromosomal breaks were identified. Chromosome no.8 was the most frequently involved in rearrangements (65 breaks), followed by chromosomes no.11 (53), no. 1 (49), no. 7 (46), no. 13 (37), no. 3 (36), no. 17 (33), and no. 20 (29). Frequent breakpoints were detected in 8q24.1 (30 breaks), 11q13 (29), 13q14 (16), 20q11.2 (14), 7q32 (13), 17q11.2 (13), 18q21 (12), 17q23 (9), 18q11.2 (9). SKY analysis identified a total of 242 chromosomal rearrangements including 190 reciprocal and non-reciprocal translocations. The recurrent combinations of chromosomal bands involved in translocations were 8q24.1 and 13q14 (3 cases), 8q24.1 and 11q13 (3), 11q13 and 17q11.2 (2), and 18q11.2 and 20q11.2 (2). Our study validated the ability of SKY to characterize in detail the chromosomal rearrangements in solid tumors and derived cell lines. Moreover, fluorescence *in situ* hybridization helped to identify the insertions, translocations, and homogeneously staining regions of *MYC* and *CCND1* gene loci.

CONCLUSION: The non-random co-localization of certain cytogenetic bands suggests the importance of chromosomal translocations in gastric carcinogenesis, by serving as landmarks for the cloning of GC causing genes.

Key words: Gastric cancer; SKY; FISH; Chromosomal translocation

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INTRODUCTION

Chromosomal aberrations are fundamental to cancer formation because they interfere with the function of oncogenes and tumor suppressor genes. Identification of recurrent chromosomal translocation may thus contribute to the cloning of cancer causing genes. Several genetic alterations associated with gastric cancer (GC) have been reported: (1) amplifications of *MYC, HST1/FGF4*, *INT2/FGF3*, *ERBB2*, *MET*, and *KSAM/FGFR2* genes[1-3], and (2) mutations of *APC*, *KRAS*, *TP53*, E-cadherin (*CDH1*), β-catenin (*CTNNB1*) and *RUNX3/PEBP2* genes[3-5]. These specific alterations have been implicated in multi-stage carcinogenesis of GC.

At the same time, previous cytogenetic studies of GC have demonstrated frequent aberrations of chromosome nos. 1, 3, 6, 7, 8, 13, 17, 20 and Y[6-9], while other chromosomes have also been found to be repeatedly involved in cytogenetic aberrations as demonstrated by recent comparative genomic hybridization (CGH) studies[10-13]. The chromosomal breakpoints identified were 1p22, 3p21, 3q23, 11p13-15, and 19p13 on primary gastric tumors[6,7,14], and 1q32, 5q11-22, 14q22, and 15q15 on human GC cell lines[15]. However, specific chromosomal translocations have not yet been identified in GC, because complete karyotypic analysis

was precluded by the complicated and cryptic nature of the rearrangements as well as the poor banding of condensed chromosomes. To overcome these limitations, multiplex fluorescence *in situ* hybridization (FISH) has been successfully used for two patients with GC[16].

To identify chromosomal translocations specific to human GC, we used multicolor spectral karyotyping (SKY) to analyze established cell lines and cancerous ascitic fluid samples and were able to identify and characterize recurrent chromosomal breakpoints and translocations in GC, several of which involved chromosomal loci of oncogenes or tumor suppressor genes.

MATERIALS AND METHODS

Cell lines and patient samples

Ten GC cell lines (KMK2, KHM14K, MKN1, MKN28, MKN45, MKN74, GT3TKB, SNU1, SNU5, and SNU16) and seven cancerous ascitic fluid samples from advanced GC (patient nos. 1-7) were the subjects of our study. SNU1, SNU5, and SNU16 were obtained from the Korean Research Institute of Bioscience and Biotechnology (Taejon, South Korea), and MKN1, MKN28, MKN45, MKN74, and GT3TKB from the RIKEN Cell Bank (Tsukuba, Japan). Two cell lines, KMK2 and KHM14K, were established at the Kumamoto University School of Medicine (Kumamoto, Japan) and their clinico-pathological features are summarized in Tables 1 and 2. Six cell lines were derived from poorly differentiated adenocarcinoma (MKN45, SNU1, SNU5, SNU16, KMK2, and KHM14K), two from moderately differentiated adenocarcinoma (MKN28 and MKN74), one from undifferentiated mucin-producing adenocarcinoma (GT3TKB), and one from adenosquamous cell carcinoma (MKN1). According to Lauren's classifications[17], seven cell lines (MKN45, SNU1, SNU5, SNU16, KMK2, KHM14K, and GT3TKB) were categorized as diffuse type, and two cell lines (MKN28 and MKN74) as intestinal type.

Primary tumors associated with carcinomatous peritonitis were histopathologically diagnosed as poorly differentiated adenocarcinoma in three patients, signet-ring cell carcinoma in two, and coexisting poorly differentiated adenocarcinoma and signet-ring cell carcinoma in two. These diagnoses indicated that all these tumors were diffuse type GC according to Lauren's classification. Ascitic fluid was aspirated with a syringe and the addition of heparin from the peritoneal cavities of seven patients with carcinomatous peritonitis. Informed consent in accordance with the institutional guidelines was obtained, for using the diagnostic material for research purposes.

Cell cultures and chromosome preparations

G-banding studies were performed as described previously[18]. Briefly, ascitic fluid was diluted in 10 mL of RPMI 1640 medium supplemented with 10% fetal calf serum at a final concentration of 1×10^6 cells/mL. The cells were cultured at 37 ℃ for 24-48 h in humidified air with 50 mL/L CO_2, exposed to colcemid (0.05 μg/mL) for 60 min, processed in 0.075 mol/L potassium chloride for 20 min, and fixed with methanol/glacial acetate (3:1). Chromosomes were stained with a Giemsa solution pretreated with trypsin, and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN 1995)[19]. The remaining chromosome pellets were stored at -20 ℃ for SKY and FISH analyses.

SKY studies

Chromosomes prepared on a slide glass were denatured and hybridized with a cocktail probe mixture for 2 d at 37 ℃. The SKY probe mixture and hybridization reagents were purchased from Applied Spectral Imaging Inc. (Carlsbad, CA, USA) and signal detection was performed according to the manufacturer's protocol. Chromosomes were counterstained with 4', 6-diaminido-2-phenylindole dihydrochloride (DAPI) combined with an anti-fade solution (Vectaschield; Vector Laboratories, Burlingame, CA, USA). Images were acquired by means of an SD200 Spectracube (Applied Spectral Imaging) mounted on an Olympus BX50-RF (Olympus, Tokyo, Japan) using a custom-designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT). With another special optical filter, the inverted DAPI images were captured in conjunction with spectral classifications as QFH band patterns for the identification of chromosomal breakpoints[20,21]. For each case, 10-20 metaphase spreads were analyzed, and karyotypes were described according to the ISCN 1995.

Double-color fluorescence in situ hybridization (DC-FISH)

Two cell lines, SNU16 and KMK2, were studied by means of DC-FISH using an oncogene and whole chromosome painting (WCP) probes. DC-FISH was performed as described previously[18]. The oncogene probe consisted of an I2 yeast artificial chromosome (YAC) clone containing *MYC* on 8q24.1 and a CPP29 cosmid containing *CCND1* on 11q13.3[22,23]. Human-specific DNA sequences were amplified from YAC by means of Alu-polymerase chain reaction[24], DNA probes were labeled by biotin-16-dUTP and digoxigenin-16-dUTP (Boehringer Mannheim Biochemicals, Mannheim, Germany). Metaphase spreads were counterstained with 0.04 μg/mL of DAPI, mounted in an anti-fade solution (Vectaschield), and observed with a BX40-RF fluorescence microscope (Olympus). Images were captured with a CCD camera (SenSys0400-G1; Photometrics Ltd, Tucson, AZ, USA).

RESULTS

Cytogenetic and SKY analysis

All established cell lines and patients with GC showed clonal karyotypic abnormalities. The modal chromosome number varied from 39 to 89. SKY detected chromosomal deletions, translocations (mainly unbalanced), inversions, insertions, dicentrics, duplication, ring chromosomes, isochromosomes, double minute chromosomes (DMs), and homogeneously staining regions (HSRs). Complex rearrangements involving more than three chromosomes were frequently encountered (Figure 1). Polysomy of chromosomes no. 16 (8 cases), no. 20 (8), no. 11 (7), and no. 9, no. 15, and no. 22 (5 each), and monosomy of chromosomes no. 5 (5 cases), and no. 4, no. 6, no. 13, no. 14, no. 15, no. 21, and no. 22 (4 each) were frequently observed. Structural rearrangements most

frequently involved chromosome no.8 (65 breaks), followed by chromosomes no. 11 (53), no. 1 (49), no. 7 (46), no. 13 (37), no. 3 (36), no. 17 (33), and no. 20 (29) (Figure 2). Chromosomal segments and bands were identified by combining SKY with DAPI banding (Figure 1). Representative karyotypes are listed in Tables 1 and 2.

A total of 630 chromosomal breaks that occurred in 177 breakpoints were identified by means of SKY analysis as shown in Figure 2 with several breakpoints highlighted: 8q24.1 (30 breaks), 11q13 (29), 13q14 (16), 20q11.2 (14), 7q32 (13), 17q11.2 (13), 18q21 (12), 17q23 (9), 18q11.2 (9), 1p22 (8), 1q32 (8), 7q22 (8), 8p11.2 (8), 8q22 (8), 9q22 (8), 10q22 (8), 20q13 (8), Xq22 (8), 7q11.2 (7), 11q23 (7), 22q11.2 (7), 3p13 (6), 3p21 (6), 3q21 (6), 4q12 (6), 9q13 (6), 13q12 (6), and 14p11.2 (6).

DC-FISH using YAC I2 and applied to two cell lines (SNU16 and KMK2) demonstrated that the chromosomal locus of the *MYC* gene (8q24.1) was involved in various types of chromosomal rearrangements including translocation, HSRs, and frequent insertion, thus resulting in gain and amplification of the gene (Figure 3). FISH using the MYC probe showed a periodic staining pattern, although we initially defined HSRs as abnormalities of chromosome no. 2 based on its SKY staining property as shown in Figure 3.

In KMK2, DC FISH with WCP 11 and cosmid CPP29 demonstrated that the chromosomal locus of the *CCND1* gene (11q13) was involved in translocations, resulting in increased copy number of the gene (Figure 4).

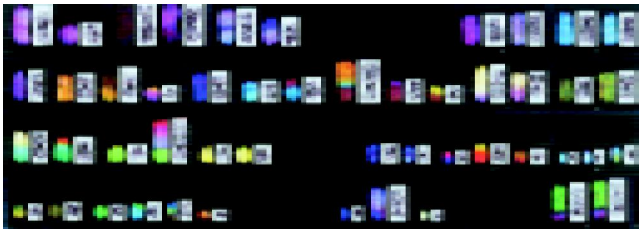

Figure 1 SKY analysis in GT3TKB. Color images are shown alongside inverted DAPI banding images.

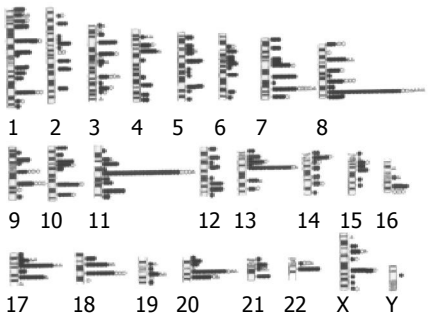

Figure 2 Assignment of breaks in 7 patients with GC and 10 established cell lines. A total of 630 breaks were identified by SKY. Closed and open circles represent diffuse type and intestinal type, respectively. Open triangle represents the breakpoints obtained from one cell line MKN1 established from a GC patient with adenosquamous cell carcinoma. Mapping analysis of breaks to the ideograms delineated two outstanding loci, 8q24.1 and 11q13. The other breakpoints highlighted are 13q14, 17q11.2, 18q21, and 20q11.2.

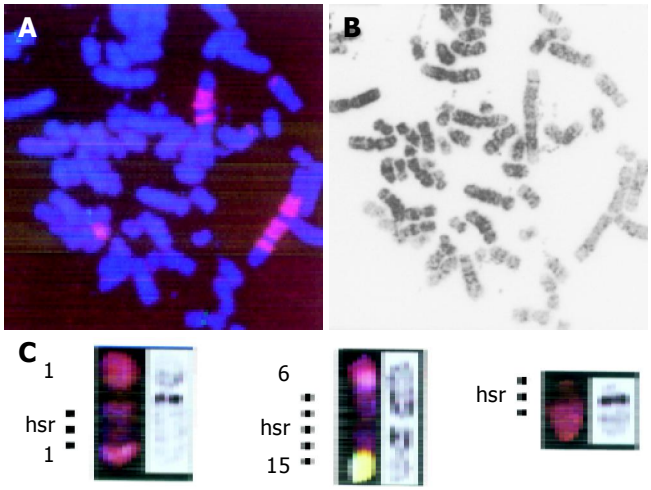

Figure 3 FISH and SKY analysis on SNU16. **A**: DC-FISH using YAC probe I2 (8q24.1, red) and cosmid probe CPP29 (11q13.3, green). I2 probe shows periodic staining pattern on HSRs, indicating amplification of *MYC* gene on HSRs but not double minute chromosomes (DMs). Three copies of *CCDN1* gene are detected; **B**: Inverted DAPI band image of the same metaphase plate shown in A; **C**: SKY findings of HSRs found in SNU16. Three HSRs were initially identified as originating from chromosome no. 2 based on their fluorescence color.

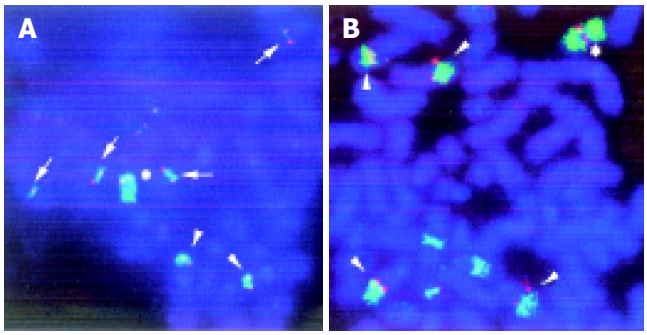

Figure 4 FISH analysis on KMK2. **A**: DC-FISH using YAC probe I2 (8q24.1, red) and WCP probe for chromosome 8 (green) demonstrates multiple insertions (arrows) and translocations (arrowheads) of *MYC* locus. Asterisk indicates a normal chromosome 8. **B**: DC-FISH using cosmid probe CPP29 (red) and WCP probe for chromosome 11 (green) demonstrates multiple translocations (arrowheads) at *CCND1* locus. Asterisk indicates a normal chromosome 11.

Identification of recurrent chromosomal translocations

A total of 242 chromosomal rearrangements including 190 translocations were identified. The four combinations of chromosomal bands repeatedly involved in translocations comprised 8q24.1 and 13q14 (SNU5, KMK2, and patient no. 6), 8q24.1 and 11q13 (SNU16, KMK2, and patient no. 1), 11q13 and 17q11.2 (KMK2 and MKN1), and 18q11.2 and 20q11.2 (GT3TKB and patient no. 7) as shown in Figure 5. Table 3 shows common translocation partners,

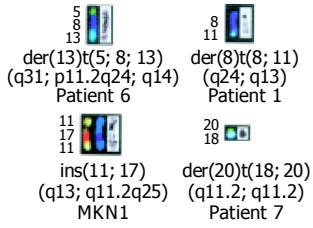

Figure 5 Partial karyotypes of recurrent translocations.

Table 1 Karyotypes according to SKY analysis on 10 cell lines derived from advanced gastric cancers

Cell line	Histologic grade[1]	Site[2]	Representative karyotype[3]
MKN45	Poor	LT	85,XX,del (1) (q32), del (1) (q32), +der (1) t (1;2) (p34;q21), +der (1) t (1;2) (p34;q21), der (2) t (2;22) (p23;q11.2), +del (2) (q23), +der (3) t (3;8) (p21.3;q24.1), +der (4) H (9qter→9q22::4p12→4q35::11p11.2→11pter), +der (4) (9qter→9 q22::4p12→4q35::11p11.2→11pter), +der (5) t (5;8) (q31;q22), +der (5) t (5;8) (q31;q22), der (6) t (1;6) (q25;q27), +der (7) t (7;12) (q32;q21), +der (7) t (7;11) (q11.1;q13), +der (7) t (7;11) (q11.1;q13), +der (7) t (7;11) (q11.1;q13), +der (7) t (7;9) (q11.2;q34),-8,-8, +9, +10, +der (10) t (8;10) (q23;p15), +der (10) t (8;10) (q23;p15), +del (11) (p11.2), +der (12) t (9;12) (p22;q24.1), +der (12) t (10;12) (q11.2;q12), +13, +13, +der (14) t (13;14) (q22;p11.2), +der (14) t (13;14) (q22;p11.2), +16, +16, +16, +der (17) t (12;17) (q22;p13), +i (17) (p11.1), +der (19) (19pter→19q13.4::7? →7?::11q23→11qter), +der (19) (19pter→19q11::8q21.2→8q24.1::20q13.1→20qter), +20, +20, +der (20) t (7;20) (q11.2;p12), +der (20) t (7;20) (q11.2;p12), +der (20) t (8;20) (p11.2;q11.2), +der (21) t (20;21) (p11.2;q11.2), +der (21) t (12;21) (p11.2;q22.3), +22, +22, +mar (6pter→6q21::20p13→20q11.2::7p11.2→7p15::8q22?8q24.1::18q21→18qter)
SNU1	Poor	AS	85,XXYY, +del (1) (p22), +del (1) (p22), -4, -5,-5, -11,-11, -13, -14, -17, -18, +20, +20, +20, +mar
SNU5	Poor	AS	74,XX,der (1) t (1;8) (p36.3;q24.1),der (1) t (X;1) (p11.4;p32), +der (1) t (X;1) (p11.4;p32), +der (2) t (2;11) (p21;q13), +der (2) t (2;11) (p21;q13), +2,-3,der (4) t (4;17) (q33;q23), +der (4) t (4;13) (q31;q32), +der (4) (4pter→4q12::13q14 →13q22::10q24→10qter), +del (5) (q15), +del (5) (p12), +5, +6, der (7) t (7;11) (q36;q13), +del (7) (q32), +del (7) (q32), +der (7) (7pter→7q22::11q13→11q23::13q12→13qter), +der (7) t (7;8) (q36;q24.1), +der (8) t (8;13) (q24.1;q14), + der (8) t (8;13) (q24.1;q14), +9, +10, +der (10) t (2;10) (p21;q24), +der (10) t (10;15) (q22;q15), +11, +11, +11, +12, der (13) (14qter→14q13::13p13→13q32::17q23→17qter), +14, +14, +der (15) t (15;22) (p13;q11.2), +16, +i (17q) (p10), +19, +20, +20, +21,-22, +hsr, +dmin
SNU16	Poor	AS	74,XX,del (1) (p13), +hsr (1) (pter→q21::hsr::q21→qter), +2, +3, +6, +6, +6, +del (7) (q22), +del (7) (q22), del (7) (q32), der (7) t (7;8) (q22;q24.1), +der (8) t (8;11) (q24.1;q13), +9,del (10) (q22), +11, +der (11) (5pter→5p13::11p11.2→11q25::17q23→17qter), + 12, +12, +13, +14, +der (15) (6pter→6p11.2→::hsr::15p11→15qter), +16, +17, +17, +18, +18, +19, +20, +20, +22, 30-50dmin
KMK2	Poor	AS	66,X,del (X) (p11.2) (q22),der (1) (20qter→20q13.1::1p36.1→1q23::8q22→8qter), der (1) (17qter→17q21::1p32→1q42::8q24.1→8qter), +der (1) (4qter→4q33::5q35→5q11.2::1p13?→q21::8q 13→8qter), +der (1) t (1;5) (q21;q32), +der (2) t (2;3) (q33;q25),t (3;5) (q25-26.2;q31), +der (3) (3pter→3q24::11q13→11q14::4p12→4pter), der (5) del (5) (p12) del (5) (q21), +der (5) (12qter→12q24.1::5p15.1→5q22::13q12→13qter), +der (5) t (5;7) (q13;q32), del (6) (q12), del (6) (q12), +del (6) (q25), +der (6) t (6;14) (q21;q22), del (7) (q22), der (7) t (7;15) (p22;q13), +der (7) t (6;7) (q15;p15), +der (7) t (7;20) (p11.2;q13), der (8) t (8;17) (p23;q11.2), der (9) (Xpter→Xp11.2::8q24.3→8q24.1::9p13→9qter), +der (9) t (3;9) (?;?), del (10) (q22), +der (10) (14q?ter→14q?24::10p13→10q21::1p13→1p34::8q24.1→8q24.3::11q13.1→11q13.5 ::7p15→7p22::15q13→15qter),der (12) (12pter→12q22::11q13.3→11q21::3q21→3qter), +der (12) (3pter→ 3p21::12p13→12q24.3::Xq13→Xq22::6p21.3→6pter), +der (12) (17qter→17q11.2::12p11.2→12q24.1::13q12 → 13qter),-13,der (13) (13pter→13q14::8q24.1→8q24.3::11q13.3→11q23::12q24.1→12qter),del (14) (q22), del (15) (q15), der (16) t (1;16) (p36.1;q22),der (17) (17pter→17q11.2:: 11q13.3→11q21::3q26.2→3qter), +der (17) t (16;17) (q22;q11.2), der (18) (22qter→22q11.2::11q21→11q13.3:: 8q24.1→8q22::18p11.2→18qter), +der (18) t (14;18) (q24;q21.3), +der (20) t (12;20) (q22;q13.1), +der (20) (20pter→20q11.2::13q12→13q14::8q24.1→8q24.3::11q13.3→11qter), +der (20) (20pter→20q11.2::13q12→13q14::8q24.1→8q24.3::11q13.3→11qter), +21, +der (22) t (5;22) (p13;q11.2)
KHM14K	Poor	LN	56,X,-Y, +1, +der (3) del (3) (p?) del (3) (q?), +der (3) (9qter?9q13::3q21→3p11::22q11.2→22qter), +der (5) (5qter →5p15::3?→3?::22q11.2→22qter),i (5p) (q10), +der (6) (6pter→6q11::5?→5?::17q23→17qter), t (8;16) (p23;q22), +der (8) t (8;20) (p11.2;q13.1), der (9) t (9;16) (p13;q12.1), +der (9) t (3;9) (q25;q13), +11, der (12) t (5;12) (q13;p13),der (13) t (3;13) (p13;q14), (14;15) (q32;q15), -15, del (15) (q24), +der (18) t (3;18) (p21.3;q21.1), +20, +20, +20,der (21) t (15;21) (q11.2-q13;p11.1),-22
GT3TKBU	Ndiffer. mucin-producing	AS	49,der (X) (Xpter→Xq28::HSR::1q32→1qter), +der (X) (Xpter→Xq28::HSR::1q32→1qter),-Y,-1, der (1) t (1;8) (p32-36.1;q24.1),der (2) t (2;4) (p12;q12-21),del (3) (p13),del (6) (q25),der (7) t (2;7) (q23;q11.2),-8,i (8q), der (9) t (9;10) (p13;q22), der (7;10) (7qter→7p22::13q13→13q11:: 10p11.2→10qter), der (10) t (8;10) (q24;p13), +der (10) t (10;15) (q11.2;q22),der (11) t (4;11) (q27;q23),der (11) t (1;11) (p13;q13),der (13) t (11;13) (q13;p11.2), der (13) t (13;17) (p11.2;p12),der (14) (14qter→14q11.2::3p11→3p26::17q11.2→17q23::3p21→3p23::17q21 →17qter), der (17) t (8;17) (q24;q11.2), der (17) t (17;19) (p11.2;q13.1), der (17) t (17;19), +del (18) (q21), +der (18) t (18;19) (q11.2;q13.1), -19,der (19) t (19;20) (p13.1;p12), +der (20) t (18;20) (q11.2;q11.2), +der (20) (8qter→8q24::20p11.2→20q11.2::2q33→2qter), +der (20) t (7;20) (q32;q11.2), der (21) t (4;21) (q12;p11.2),-22
MKN28	Moderate	LN	47, X, -X, del (1) (p22), +der (1) t (X;1) (q22-24;q32), del (2) (q13), del (2) (q13), +der (2) t (2;18) (p23;q21.3), der (3) del (3) (p13p25) del (3) (q21q25), +der (3) del (3) (p12) del (3) (q21q26.2), -4, -5, -5, -7, der (7) t (7;8) (q36;q22), -8, der (8) t (8;10) (p23;q11.2), -10, -13, der (14) t (7;14) (q11.2;q11.2), der (14) t (1;14) (q42;q24), +15, +der (16) t (13;16) (q12;q23-24), +der (19) t (11;19) (q13;q13.3), der (20) (20pter→20q13.1::8q13→8q24::7q32→7qter), +der (20) t (18;20) (q21.1;q11.2), +21, +21

MKN74	Moderate	LT	39, X, +der (X) (15qter→15q11.2::Xp11.2→Xq28::8q24.1→8qter), +der (X) t (X;7) (q13;q32), -Y, der (1) (9qter→9q32::1p34→1q32::18q21→18qter), +2, -3, der (4) t (4;6) (p12;p21.3), -5, der (7) (7pter→7q32::8q22→8q24.1::9q22→9qter), der (7) t (7;8) (q32;q22), der (8) t (8;10) (p23;q22), der (9) t (9;11) (q22;q13), -9, -10, +del (11) (q13), -12, -13, der (14) t (9;14) (q13;p11.2), -14, -15, der (16) t (9;16) (q13;q24), +der (16) t (9;16) (q13;q24) del (9) (q22), der (18) t (2;18) (?;q23), der (20) t (13;20) (q32;q11.1), -20, -21, -21, der (22) t (10;22) (q24;p11.2), +der (22) t (13;22) (q14;p11.2)
MKN1	Adenosqu amous carcinoma	LN	56, X, +inv (X) (p11.2p22.1), -Y, +der (1) t (1;8) (p36.1;q24.1), +der (1) t (1;8) (p36.1;q24.1), der (3) t (3;5) (p11;p12), der (3) t (3;20) (q27;q13.1), +der (3) t (3;7) (p26;q32.1), -5, -5, -6, +7, +7, +7, +7, del (8) (q24.1), +del (8) (q24.1), +del (8) (p11.2), +del (8) (p11.2), -9, -9, +ins (11;17) (q13;q11.2q25), -12, +del (13) (q14q22), der (15) t (4;15) (q12;p11.2), -15, +del (16) (q12.1), +del (16) (p11.2), +16, der (17) t (4;17) (p15.2;p11.2), der (17) t (4;17) (p15.2;p11.2), del (17) (q11.2), +der (18) t (3;18) (q21;q11.2), del (18) (q11.2), del (19) (p13.1), der (19) t (19;21) (q13.1;q11.2), +der (19) t (19;21) (q13.1;q11.2), +del (20) (q11.2), +der (20) t (17;20) (q23;q11.2), der (22) t (11;22) (p13;p11.2)

[1]Poor: poorly differentiated adenocarcinoma; Moderate, moderately differentiated adenocarcinoma. [2]ST: stomach; LT: metastatic liver tumor; AS: ascitic fluid; LN: lymph node. [3]Representative karyotypes are defined based on G-banding and SKY findings, and described according to ISCN1995.

Table 2 Karyotypes according to SKY analysis on 7 patients with advanced gastric cancer and carcinomatous peritonitis

Case number.	Age/Sex	Origin[1]	Histologic grade[2]	G-banding and SKY
1	52/M	AS	Poor	89, XX, der (1) t (1;13) (q44;q14), t (1;2) (p36.3;p11.1), +der (1) t (1;2), +der (1) t (1;8) (p22;q24), +der (1) t (1;8) (p22;q24), +der (1) t (1;12) (q32;q13) del (1) (p32), +2, +2, +3, +4, +4, +del (5) (q13), +6, +6, +6, +7, +7, +7, der (8;8) (8pter→8q24.1::8p23→8q24.1::17q23→17qter), +der (8) t (8;11) (q24.1;q13), +del (8) (p11.2), +del (8) (p11.2), +9, +9, +i (9q), del (10) (q24), +der (10) t (8;10) (q24.1;q11.2), +der (10) t (8;10) (q24.1;q11.2), +inv (10) (p15.1q21), +11, +11, +12, +14, i (15q), i (15q), +16, +16, +17, del (18) (q11.2), +der (18) t (10;18) (p11.2;p11.2), +der (18) t (10;18) (p11.2;p11.2), +der (18) t (10;18) (q22;q21), +der (18) t (10;18) (q22;q21), +19, +19, +20, +20, +20, +20, +20, +20, -21, -21, +22
2	72/M	AS	Poor	47, XY, +X, -3, del (13) (q14), +mar
3	63/M	AS	Poor	54, der (X) t (X;3) (q24;q21), Y, +2, +del (4) (q21), +der (4) (4p15→4q12::17q11.2→17qter), +del (5) (q13), der (6) t (Y;6) (q11.2;p21.1), t (7;16) (q36;q22), der (11) t (9;11) (q13;q13), +del (11) (q13), +11, del (12) (p11.2), der (13) t (8;13) (q13;q14), +15, +16, -21, -21, +22, +22
4	56/M	AS	Signet	46, X, -Y, +der (1) t (?;1;?) (?::1p13→1q21::?), t (2;20;8) (p11.2;q11.2;q24.1), t (2;20;8) (p11.2;q11.2;q24.1), +2, +2, der (3) (3qter→3p13::7?→7?::11q13→11qter), del (3) (p23) (q25), -4, -4, +del (5) (q13), +del (5) (q11.2), del (6) (q11), +der (6) (6p21.1→6q21::18q11.2→18qter), dic (7;11) (q11.2;p15), del (9) (p11), del (10) (p11.2), del (10) (p11.2), +10, der (11) t (?;11;?) (?::11p13→11q23::?), der (11) (2pter→2p11.2::11q12→11p11.2::7q11.2→7q32::17q23→17qter), der (11;20) (20pter→20q11.2::11p13→11q23::11q13→11q14::7q31→7q36::13q14→13qter), del (12) (q13), del (12) (q22), der (13) t (X;13) (p11.2;p11.2), der (13) (3pter→3p13::13p13→13q14::18q21→18qter), -14, -15, del (16) (q22), add (17) (p11.2), add (18) (q21.3), -18, -19, +20, der (21) t (17;21) (q23;q22), der (21) (7pter→7p11.2::21p13→21q22.1::hsr::21q22.1→21qter), -22
5	55/M	AS	Signet	50, add (X) (p22.3), Y, +8, +9, +16, +18
6	37/M	AS	Poor	69, der (X) (Xpter→Xq22::18q11.2→18q21::4q31.1→4qter),
			Signet	der (X) (Xpter→Xq22::18q11.2→18q21::4q31.1→4qter), -Y, +der (1) t (1;12) (q25;q13), +del (1) (p22), del (2) (q23), del (2) (q23), +der (2) t (1;2) (q23;q21), +der (2) t (1;2) (q23;q21), del (3) (p21), +del (3) (p13), +del (3) (q11.2), +5, +5, der (6) t (6;18) (q12;q11.2), -6, +7, +7, +der (8) (5qter→5q31::8p11.2→8q24.1::13q14→13qter), +der (8) (5qter→5q31::8p11.2→8q24.1::13q14→13qter), +der (10) t (4;10) (q12;q22), +der (10) t (4;10) (q12;q22), +der (11) t (7;11) (q22;q23), +der (11) t (11;17) (q25;q11.2), +12, der (13) t (6;13) (p21.1;p11.2), -13, der (14) t (14;17) (p11.2;q11.2), der (14) t (14;17) (p11.2;q11.2), +15, +15, +16, +16, der (17) t (X;17) (q22;q11.2), der (17) t (X;17) (q22;q11.2), +18, +18, +20, +21, +der (21) t (X;21) (q22;p11.2)
7	73/F	AS	Poor	67, X, del (X) (q26), der (1) (1qter→1p22::11?→11?::8?→8?::22q11.2→22qter), der (1) (1?→1?::15?
			Signet	→15?::1?→1qter), der (1) (9pter→9p13::1p12→1q32::9q22→9qter), -2, -5, +der (7) t (7;9) (q32;q22), +der (7) t (7;9) (q32;q22), +der (7) t (7;20) (q22;q11.2), +der (7) t (7;20) (q22;q11.2), t (8;14) (q13;p13), +der (8) t (8;14), t (6;8) (p11.2;p21), +8, -9, +der (10) t (2;10) (p13;q24), +11, -12, +der (14) (20qter→20q13.1::14p11.2→14q24::11q13→11qter), +der (14) t (3;14) (p21;q13), +14, +15, +16, +17, +18, +18, +18, +18, +der (18) t (18;20) (q11.2;q11.2), +19, +20, +21, +21, +22, +der (22) t (1;22) (p22;p11.2)

[1]AS: ascitic fluid. [2]Poor: Poorly differentiated adenocarcinoma; Signet: signet ring cell carcinoma.

Table 3 Recurrent breakpoints and partners in gastric cancer cells defined by SKY analysis

Translocation breakpont	Number of breakpoints	Candidate genes	Partner breakpoints involved in chromsomal translocations or deletions[1]	
			Common	Single
8q24.1	30	MYC	11q13 [3], 13q14 [4]	1p22, 1p32-36.1, 1p34, 1p36.1, 1p36.3, 1q42, 2p11.2, 3p21.3, 7q22, 7q32, 7q36, 8p23, 9p13, 9q22, 10p13, 10q11.2, 17q11.2, 17q23, 18q21, 20p11.2, 20q11.2, 20q13.1, Xq28, deletion
11q13	26	FGFR4	8q24.1 [3], 8q24.3 [3],	1p13, 2p21, 7p15, 7q11.1, 7q22, 7q36, 7?, 9q13, 9q22, 11q23, 12q22, 13p11.2, 14q24, 17q25, 19q13.3
		FGF3 CCND1	3q24-25 [2], 17q11.2 [2], deletion [2]	
20q11.2	14	Unknown	18q11.2 [2]	2p11.2, 2q33, 7p11.2, 7q22, 7q32, 8p11.2, 8q24, 11p13, 13q12, 17q23, 18q21.1, deletion
1p32-36.3	12	RUNX3	8q24 [4]	2p11.1, 2q21, 9q32, 16q22, 17q21, 20q13.1, Xp11.4, deletion
13q14	12	RB	8q24.1 [3]	1q44, 3p13, 4q12, 7q36, 8q13, 13q22, 18q21, 22p11.2, deletion
17q11.2	12	ERBB2	11q13 [2]	3p26, 4q12, 8p23, 8q24, 11q25, 12p11.2, 14p11.2, 16q22, Xq22, deletion
7q32	11	MET	deletion [2]	3p26, 5q13, 8q22, 8q24, 9q22, 12q21, 17q23, 20q11.2, Xq13
18q21	11	BCL2	None	1q32, 2p23, 3p21.3, 4q31.1, 8q24.1, 10q22, 13q14, 14q24, 20q11.2, deletion
17q23	10	Unknown	11q25 [2]	3p21, 4q33, 5?, 7q32, 8q24.1, 13q32, 20q11.2, 21q22
10q22	9	KSAM	deletion [2]	4q12, 6p25, 8p23, 9p13, 15q15, 15q22, 18q21
18q11.2	9	Unknown	20q11.2 [2], deletion [2]	3q21, 6q12, 6q21, 19q13.1, Xq22

[1]The number of breaks are indicated.

some of which were previously undetectable because of the structurally complicated chromosomal rearrangements. The same der(20)t(18;20)(q11.2;q11.2) was found in two cases (GT3TKB and patient no. 7; Figure 3), and chromosome deletions of del(7)(q32), del(10)(q22), del(11)(q13), and del (18)(q11.2) were detected in two cases each.

DISCUSSION

SKY analysis proved to be capable of identifying 630 breaks and 242 rearrangements of chromosomes in 10 established cell lines and seven cancerous ascitic fluid samples of GC, as well as of identifying recurrent breakpoints. Chromosomal rearrangements frequently involved well-known oncogene and tumor suppressor gene loci that may be associated with gastric carcinogenesis, such as 8q24.1 (*MYC*), 11q13 (*HST1/FGF4, INT2/FGF3, and CCND1*), 7q32 (*MET*), 13q14 (*RB*), 17q11.2 (adjacent to the *ERBB2* locus), 18q21 (*DCC* and *BCL2*), 7q22 (*MET*), 3p21 (*CTNNB1*), and 16q22 (*CDH1*). The other breakpoints that were detected more than eight times were 20q11.2 (14 breaks), 17q23 (9), 18q11.2 (9), 1p22 (8), 1q32 (8), 8p11.2 (8), 8q22 (8), 9q22 (8), 10q22 (8), 20q13 (8), and Xq22 (8). However, it is not clear which breakpoints represent key genes during the earlier stages of tumorigenesis in this lineage, because when the entire tumor was examined in our study, it was already highly progressed and in the case of cell lines, they were further selected for liver culture conditions.

Two distinct breakpoints, which were not noted as unique occurrences by G-banding, 8q24.1 (30 breaks, 4.8% of 630 breaks) and 11q13 (29, 4.6%), were identified by a combination of SKY and DAPI banding analysis. The currently most likely candidate at 8q24 is the *MYC* gene, because it was frequently amplified and overexpressed in advanced GC, particularly in patients with carcinomatous peritonitis or distant metastasis[25,26]. The DC-FISH analysis used in our study showed that the *MYC* locus was involved in amplification resulting from the HSR as well as in multiple chromosomal translocations, insertions, and duplications in KMK2 (Figures 3 and 4). However, in SNU16, the *MYC* locus was amplified in HSRs but not on DMs, suggesting that other genes, for example the *K-sam* gene, may be involved in DMs (Figure 3)[27].

The second common breakpoint of 11q13 contains a variety of genes associated with cell proliferation and differentiation such as *HST1/FGF4, INT2/FGF3, CCND1, SEA, MYEOV*, and *SPA1*[28,29]. It has been demonstrated that *HST1/FGF4* is co-amplified with *INT2/FGF3* in human GC, although amplification of *FGF4* and *FGF3* does not correlate with mRNA overexpression[30], while high-level amplification of 11q13 has been frequently detected in CGH analysis[12,13]. All these genes are assigned to band 11q13.3. Band 11q13 may thus span a region approximately 25 Mb in size as estimated from both the physical length of 11q (86 Mb) and the number and size of the bands, which are divided into five sub-bands including three light bands (q13.1, q13.3, and q13.5). Since it is difficult to distinguish these subregions in a karyotype of 400 bands, 11q13.1 and 11q13.5 also warrant molecular dissection for the identification of target genes.

Combining SKY and DAPI banding analysis led to the identification of recurrent co-localization of the chromosomal bands involved in translocations: 8q24.1 and 13q14 (3 cases), 8q24.1 and 11q13 (3), 11q13 and 17q11.2 (2), and 18q11.2 and 20q11.2 (2). These translocations used to be frequently undetectable because of the complicated rearrangements of chromosomes. In most cases, each co-localized breakpoint contains chromosomal segments to which oncogenes or tumor suppressor genes have been assigned.

In conclusion, SKY analysis identified the frequently occurring breakpoints 8q24.1, 11q13, 20q11.1-13.1, and 13q14 and the recurrent translocations 8q24.1 and 13q14, 8q24.1 and 11q13, 11q13 and 17q11.2, and 18q11.2 and 20q11.2. SKY thus proved to be extremely useful for a comprehensive analysis of chromosomal translocations in GC and derived cell lines. The chromosomal breakpoints defined in our study may well contain critical genes which are involved in multistage carcinogenesis of GC and thus can serve as landmarks for crucial regions that warrant molecular dissection.

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