DIRECT CHROMOSOME ANALYSIS AND FISH DETECTION OF PRIMARY GASTRIC CANCER

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ABSTRACT

Objective: To investigate chromosome aberrations and their role in the genesis and development of primary gastric cancer. Methods: An improved, direct chromosome preparation from solid tumors was adopted for G-banding analysis followed by FISH on decolored G-banding chromosomes so that chromosome aberrations could be confirmed at DNA level. Results: A total of 28 primary gastric cancer specimens were studies. Case 1 and case 2 had simple chromosome numerical changes: 49, XY, +2, +8, +9 and 48, +8, +20, respectively. All but case 1 and 2 had complicated chromosome abnormalities. Chromosome structural of frequent occurrence involved del(7q)(21/26), del(3p)(14/26), del(lp)(11/26) and del(17p)(10/26). The chromosome abnormalities could be simple and complicated. In former, numerical changes involving 1 to 3 chromosome could be observed. Trisomies 8 and 9 might represent a cytogenetic subgroup of primary gastric cancer. In the later, the del(7q) was the most consistent aberration. 7q32-qter was the commonly lost segment. Conclusion: Numerical and structural alterations of chromosomes are present in primary gastric cancer. Del(7q) is one of the structural change characteristic of primary gastric cancer. In the 7q32-qter fragment, a tumor suppressor gene probably exists and it may have close relation to the genesis and progression of gastric cancer.

Key words: Gastric cancer, Chromosome changes, Cytogenetics, FISH

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The cytogenetic studies about primary gastric cancer were relatively few. The chief difficulty was poor banding quality in short-term culture, and the analysable karyotypes were hardly obtained. On the other hand, the chromosome changes in gastric cancer were very complicated, some marked chromosomes were hardly distinguished often. So far, no consistent specific chromosome changes had been verified. We reported here the direct chromosome analysis of 28 primary gastric cancers and the detections of fluorescence *in situ* hybridization (FISH) performed on decolored G-banding chromosomes. These results revealed some specific chromosome changes in primary gastric carcinoma.

MATERIALS AND METHODS

Clinical Samples

Twenty-eight cases of primary gastric carcinoma were obtained from the Tumor Hospital, Harbin Medical University. The patients had not received any preoperative chemotherapy or radiotherapy.

Chromosome Preparation

Tumor materials were processed directly for cytogenetic studies with an improved method.[1] Briefly, about 1 cm^3 tumor specimen was transported to the cytogenetic laboratory in RPMI 1640 medium without serum as soon as the tumor tissue was resected. The specimen was finely minced with scissors after separated from non-neoplastic and necrotic tissues. The cell suspension and remaining tissue fragments were incubated with colchicine (final concentration 1 μg/ml) at 37°C in a water bath for 1 h, and were then treated hypotonically with 0.4% KCl and 0.4% sodium citrate (1:1) twice, ten minutes each time. The cells were fixed in

Carnoy's fixative (Methanol 3: Acetic acid 1) for only ten minutes before being dropped on slides. Slides were air-dried two to three days, then GTG-bandings were performed.

Karyotype Analysis

Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN).[2] Clonality was defined by the detection of two cells with gain of a given chromosome or the same structural abnormality, or three cells with loss of a given chromosome.

Fluorescence *in situ* Hybridization (FISH)

After GTG-banding, several metaphase spreads with good banding were photographed. The slide was then destained by washing in absolute methanol twice for 10 min each, rinsed in 70% and 85% ethanol for 1 min each, followed by washing in 3:1 methanol:glacial acetic acid for 10 min, and air dried. Then, the slide was washed with 1% formaldehyde in 1×PBS/$MgCl_2$ for 10 min, two washes in PBS for 5 min each, and an ethanol wash series and then air dried. FISH with biotin-labeled centromeric probes (Oncor, Inc.) or chromosome painting probes (NHGRI of NIH) was performed following procedures described by Pinkel et at..[3] Briefly, the slide with metaphase spreads was denatured in 70% formamide/2×SSC at 75°C for 2 min. For each hybridization, about 100 ng of chromosome painting probes in 10 μl hybridization mixture (containing 55% formamide, 2×SSC, and 1 μg human cot-I DNA (BRI), or 100 ng of centromeric probes in 10 μl hybridization mixture (containing 55% formamide, 2×SSC) was used, and denatured at 76°C for 8 min, and then hybridized with target DNA at 37°C in a moist chamber overnight. The slide was then washed three times in 50% formamide/2×SSC at 45°C for 3 min each. The hybridization signals of the probes were detected by two stages of FITC-conjugated avidin (Vector) and amplified with one layer of anti-avidin antibody (Vector). The slide was counterstained with 0.5 μg/ml propidium iodide in antifade solution, and was examined with a Zeiss Axioskop 20 microscope and photographed.

RESULTS

Chromosome Changes of Primary Gastric Cancer

Twenty-eight cases of primary gastric cancer were studied with direct harvesting before patients received any treatment. Table 1 shows the clinic pathologic data of 28 cases with detailed karyotypes. Chromosome counting showed that 18 cases were in diploid range, six in triploid range, and four in tetraploid range. Chromosome gains and losses involved most chromosomes. Cases 1 and 2 had simple chromosome changes: 49, XY, +2, +8, +9 (Figure 1), and 48, XX, +8, +20. The trisomy 8 and 9 in case 1 were tested by FISH, and showed three strong hybridization signals. The trisomy 8 in case 2 were tested by FISH, and showed three strong hybridization signals (Figure 2). Other twenty-six primary gastric cancers had complicated numerical and structural chromosome aberrations. The structural abnormalities which occurred most frequently in 26 gastric cancers were del(7q)(21/26), del(3p) (14/26), del(lp) (11/26) and del(7q) (10/26). The frequent structural aberrations were terminal deletions and unbalanced translocations. (Figure 3). The structural aberrations were tested by FISH, and showed strong hybridization signals (Figure 4).

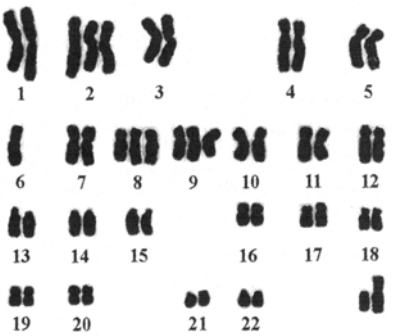

Fig. 1. G-banding karyotype from case 1. 49, XY, +2, +8, +9. The loss of chromosome 6 was considered to be random.

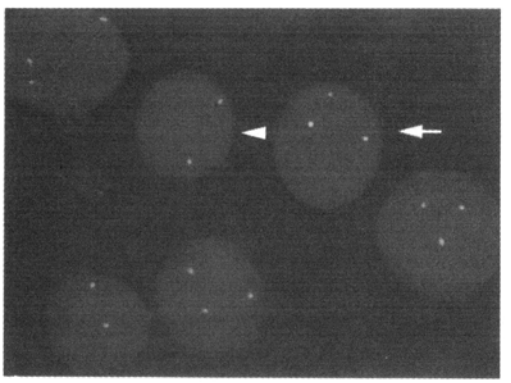

Fig. 2. FISH of biotin-labeled centromeric probes of chromosome 8 on interphase nucleus from case 2. The two yellowish-green hybridization signals (arrow, ▶) in two normal cell; The three yellowish-green hybridization signals (arrow, ➔) in the four tumor cells show trisomy 8.

Table 1. Clinical, histopathologic, and cytogenetic findings in twenty-eight cases of primary gastric cancer

Case No.	Histology	Degree of Differentiation	Chromosome number	Changes of clonality chromosome
1	Mucin-producing adenocarcinoma	Poor	49	+2, +8, +9
2	Adenocarcinoma	Poor	48	+8, +20
3	Adenocarcinoma	Moderate	38	del(1)(p22), add(1)(p11), -2, del(3)(p13), del(5)(p12), +7, -10, del(10)(q24), -11, add(12)(p11), add(13)(p11), add(14)(p11), -15, -17, -18, -21, -22
4	Adenocarcinoma	Moderate	80–81	del(1)(p13), i(1q), +3, add(3)(p11), +5, +7, del(7)(q31), add(7)(p22), del(10)(q23)×4, add(11)(p13), add(12)(p11), +13, der(14)t(14q15q), +16, +17, i(17q), +19
5	Adenocarcinoma	Moderate	52–54	-4, del(7)(q22), -8, add(9)(q34), add(10)(q24), +12, -14, +15, -16, i(17q), +19, +20
6	Signet-ring adenocarcinoma	Poor	38–42	-X, -Y, del(1)(q32), der(13)t(13q14q), -18, -20
7	Adenocarcinoma	Moderate	54–58	-Y, del(3)(p24), del(6)(q15), i(6p), +7, del(7)(q22)+8, +10, +13, i(15q), -17, i(17q), -21
8	Adenocarcinoma	Poor	62	add(1)(q32), del(1)(p13), del(3)(q21), add(5)(q31), +6, del(7)(q22)×2,, +10, add(12)(p11), -14, -18, -19, -21
9	Adenocarcinoma	Moderate	55	-Y, +1, +2, +3, del(3)(p21), i(6p), add(6)(q27), add(7)(q11), -14, -20, -21, -22
10	Adenocarcinoma	Poor	58–62	+X, add(3)(p11), del(7)(q22), del(7)(q22), +11, +15
11	Adenocarcinoma	Poor	50–53	del(1)(p22), +2, del(3)(p12), +6, +7, add(7)(q22), +8, -14, -17, -18, -21, -22
12	Adenocarcinoma	Moderate	64–68	-Y, add(2)(q33), del(3)(p12)×2, +5, +6, del(6)(q21), del(7)(q32)×2, -8, -11, add(12)(p11), +13, +14, +18, +20
13	Adenocarcinoma	Poor	74–78	add(1)(p32), +2, del(3)(p21), del(3)(q21), +4, +6, add(6)(q11), del(7)(q22), add(7)(q11), del(8)(p21), +11, add(11)(q11), der(12)(12q15q)×2, der(14)t(14q15q), +16, +18
14	Adenocarcinoma	Poor	78–83	-X, -Y, del(3)(p21), +5, -7, add(7)(p11), +8, +11, add(12)(p11), +13, i(17q), +19, +21, -22
15	Adenocarcinoma	Poor	63–66	+1, add(1)(p11), +8, add(11)(q11), der(13)t(13q14q), +16, i(17q), +18
16	Adenocarcinoma	Poor	55–57	del(X)(q24), del(1)(p11), +2, de(3)(p21),add(5)(q35), del(7)(q22), +8, +12, -16, -18, -21
17	Adenocarcinoma	Poor	44–48	-X, del(X)(q26), del(1)(p13), del(7)(q22)×2, -17
18	Adenocarcinoma	Poor	54–58	-Y, +2, add(2)(q33)×2, del(3)(q12), -6, del(7)(q12), add(7)(q32), +8, +13, i(17q)
19	Adenocarcinoma	Moderate	90–93	del(1)(p21), +5, +6, +7, del(7)(q34), +8, -10,del(10)(q22), +13, add(14)(p11)×3, +16, add(17)(p11)×2, +19, -20
20	Adenocarcinoma	Moderate	63–65	-Y, del(6)(q15), i(6p), +7, del(7)(q32), +10, +13, i(15q), -17, i(17q), -21
21	Adenocarcinoma	Poor	45	add(1)(p11), +4, -6, del(7)(q22), i(8q), +19
22	Adenocarcinoma	Moderate	91–93	add(7)(q32), add(7)(q21), +7, del(10)(q22), +13, add(14)(p11)×3, +16, add(17)(p11)×2, ring[?der(3)]
23	Adenocarcinoma	Poor	64–72	-X, -Y, del(3)(p21), add(7)(q11), -7, +13, +16, +19, +20
24	Adenocarcinoma	Poor	48–52	add(1)(p13), add(2)(p23), -5, add(6)(q25), i(7q), +13, -14, +16, -18, -19, -21, -22
25	Adenocarcinoma	Poor	74–78	+X, +2, add(3)(p25), +4, add(5)(q23), +6, +7, del(7)(q32)×2, +10, +11, +12, +16, +19, +19, +20, +21
26	Adenocarcinoma	Poor	44–48	del(X)(q22), -Y, +4, del(6)(q23), del(7)(q34), -8, -11, -12, -14, add(15)(p11), add(15)(q26)
27	Adenocarcinoma	Poor	55–59	-Y, add(2)(q11)×2, +2, del(3)(p12), -6, add(7)(q22), +13
28	Adenocarcinoma	Poor	44–50	del(3)(q25), -6, +7, add(7)(q32), -18, -19, -20

FISH Performed on the Decolored Conventional G-banding Chromosomes

The conventional (G-banding analysis on gastric cancer chromosomes was performed at first. We elementarily knew chromosome changes in gastric cancer. According to the results of chromosome changes, we selected suitable probes and performed FISH on the decolored G-banding chromosomes so that the chromosome aberrations could be confirmed at DNA level. For example, the G-banding karyotype of case 12 involved del (7) (q32-qter). FISH was performed on the decolored G-banding chromosomes case 12 by use of 7q painting probe, and the del (7) (q32-qter) was confirmed at DNA level (Figure 4).

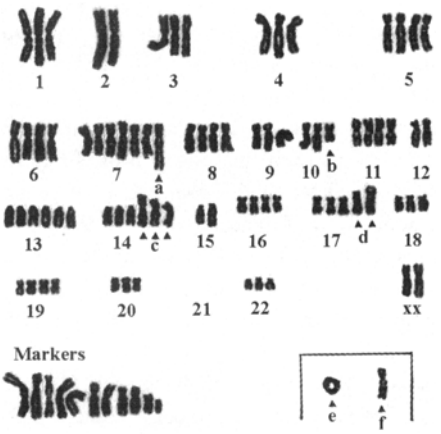

Fig. 3. G-banding karyotype from case 22. The clonal chromosomal rearrangements identified involve: (a) add(7) (q22); (b) del(10)(q22); (c) add(14)(p11)×3; (d) add(17) (p11)×2. Inset: clonal changes from other metaphases of this case, (e) ring chromosome; (f) add(7)(q2l).

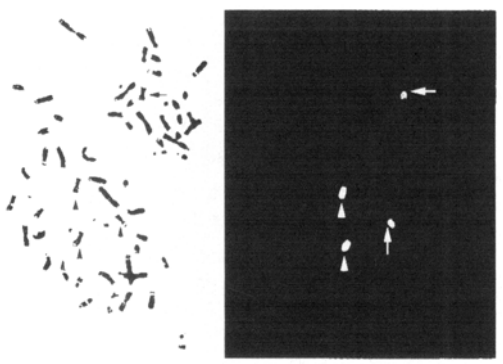

Fig. 4. The contrast analysis of FISH of biotin-labeled chromosome painting probe of 7q and G-banding on metaphase spread from case 12. The photograph of the metaphase spread show two normal 7q (arrow, ▶) and two abnormal 7q (arrow, →). The structural changes of the two abnormal 7q by G-banding analysis involved del(7)(q32)(M3) and del(7)(q32).

DISCUSSION

The Simple Chromosome Changes in Primary Gastric Cancer

So far, the karyotypes of simple chromosome numerical changes in primary gastric cancer had been reported in six cases:

(1) 48, X, +X, 12/47, XX, +X[1]; (2) 47, XX, +X/48, XX, +X, +X[2]; (3) 49, XY, +8, +9, +19[3]; (4) 48, XX, +8, +19[2]; (5) 49, XX, +8, +9, +12[4]; (6) 47, XX, +8[5]. In present study, case 1 and case 2 also had similar changes, so chromosome changes in primary gastric tumors might be divided into the simple and the complicated types. The simple type involved 1 to 3 chromosome changes and the most commonly observed abnormalities were trisomy. Trisomy 8 and 9 might represent a cytogenetic subgroup of gastric cancer. In addition, although case 1 and case 2 had simple chromosome numerical changes, both of them had poor degree of differentiation (Table 1), and their clinical progression was very rapid. Thus, simple chromosome changes were not consistent with clinical progression. The pathogenesis of the simple types in primary gastric cancer remained to be studied further.

The Complicated Chromosome Structural Changes in Primary Gastric Cancer.

Chromosome changes in the other twenty-six cases were complicated. Among them, twenty-one cases contained del(7q) or unbalanced translocations resulting in del(7q), and the commonly lost segment was 7q32-qter. This was the most consistent changes in the present study. In previous cytogenetic studies of primary gastric cancers, del(7q) was frequently reported: del(7q)(4/5) by Ochi,[1] del(7q)(5/8) by Xiao,[2] del(7q)(4/9) by Rodriguez[6] and del(7q)(4/6) by Rao.[7] Therefore del(7q) might be regarded as one of the specific chromosomal lesions of primary gastric cancer. Recently, the study results of molecular genetics in gastric cancer indicated that LOH of gastric cancer frequently occur in 7q. LOH studies of gastric carcinomas also suggested that chromosome region 7q31-tqer may contained a candidate suppressor gene for cancer.[8,9] Therefore, the chromosome fragment 7q32-tqer might contain a tumor suppressor gene for the genesis and progression of gastric cancer. Whether del(7q) in gastric cancer possibly results in the function loss of a tumor suppressor gene is worthy to be studied further.

Del(3p) was observed in fourteen cases in present study. The commonly lost segment was 3p2l-pter. The del(3p) in gastric cancer were frequently reported previously.[2,5,6] LOH study of gastric cancer also suggested that 3p existed the tumor suppressor gene

related gastric cancer.[10,11] Furthermore, some tumor suppressor genes, such as FHIT, PTPG and CTNNB, were located on 3p21, 3p22 and 3p25. The locations of these suppressor genes were all in the common loss segment of 3p in present study. Whether del(3p) possibly resulted in loss of one or several tumor suppressor genes related to the pathogenesis of gastric cancer remained to be deciphered.

Other frequent structural changes in present study involved del(1p) and del(17p). Del(1p) was observed in eleven cases. The common lost segment was 1p32-pter. Del(17) was observed in ten cases, and the common lost segment was 17p13-pter. Previous studies of gastric cancer also revealed del(1p).[6, 12] Recently, LOH studies of 26 primary gastric cancers indicate that chromosome fragment 1p34–p35 may contained a condidate suppressor gene.[13] The genes for the type II (p75) tumor necrosis factor receptor (TNF-RII) and LAR were located on 1p36.2–p36.3 and 1p32, respectively. The locations of the two genes were in the common lost segment of 1p in present study. Otherwise, the tumor suppress genes Tp53 was located in 17p13.1. LOH studies revealed that the approximately sixty percent primary gastric cancer was involved the loss of Tp53. Whether del(1p) and del(17p) possibly resulted in loss of tumor suppressor genes related to the pathogenesis of gastric cancers remained to be elucidated.

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