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Chromosome Abnormalities of Gastric Cancer Detected in Cancerous Effusions

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The chromosomes were examined in cancerous effusions obtained from 7 patients with gastric cancer. In 1 patient, the modal chromosome number was 46 with a normal chromosome complement in the majority of the cells examined. The other 6 patients revealed numerical and structural aberrations. Among the structural rearrangements detected, chromosome 3 was involved in the short arm and in the long arm, each in three patients; bands p25, q21, q23, and q27 were recurrently involved and a band q23 was lost in 3 patients. Rearrangements of chromosome 5 in the long arm and of chromosome 17 at or near the centromere were also observed in three patients each. Gains of chromosome 7 was seen in 5 patients and of chromosome 13 in 4. Some of these alterations may be specific for gastric cancer.

Key words: Chromosomes — Human — Gastric cancer — Effusion

In human solid tumors, several characteristic and specific chromosomal changes have been described.[1-3] These aberrations are assumed to be involved in the process of initiation or progression of the tumor. There may be three types of chromosome abnormalities in cancer cells: primary changes that are specific for one histopathologic type of tumor; secondary nonrandom changes; and random changes.[4] In a summary report by Hecht concerning chromosome rearrangements in solid tumors,[3] 15 specific rearrangements are listed that are assumed to be characteristic for certain types of cancer and 51 additional rearrangements that may be not specific but are recurrently detected; these include translocations, deletions, isochromosomes, duplications, and inversions. In the search for suppressor gene losses, allelic losses of the same sites were detected by chromosome analysis in several tumors, but the results are contradictory in other tumors.[5] In gastric cancer, technical limitations exist, and enough results are not yet available on the chromosome constitution. Structural and/or numerical abnormalities of chromosomes 8 and 9 were recurrently observed in a series of investigations reported by Ferti-Passantonopoulou *et al.*[6] In another series, Ochi *et al.*[7] described 67 numerical and 83 structural chromosome abnormalities in 5 patients. Among these changes a missing Y and alterations of the short arm of chromosomes 1, 3, and 19 were frequent. Studies of RFLP suggested the loss of alleles in chromosomes 1, 5, 12, and 13.[8-10]

We examined the chromosomes in cancerous effusions obtained from 7 patients with gastric cancer. Among 6 patients in whom the karyotypes were determined, a number of numerical and structural abnormalities were detected. The results are analyzed herein together with those previously reported in order to identify specific or recurrent chromosomal changes for gastric cancer.

MATERIALS AND METHODS

Patients The series comprises 7 patients (2 males and 5 females) with gastric cancer (Table I). The macroscopic type of gastric cancer[11] was IIc+III type early cancer in 2 patients, Borrmann 3 type advanced cancer in 1, and Borrmann 4 type in 4. The 2 patients with IIc+III type gastric cancer showed invasion of cancer cells beyond the muscularis propria and the cancer was advanced cancer histologically. Five patients underwent surgical treatment but resection was successfully done in only 3 of these patients. The remaining 2 patients were not operated. Cytostatic chemotherapy was carried out in these patients. Mitomycin C and fluorinated pyrimidines (5-FU, FT-207, or UFT) were given to every patient intravenously or orally. In patient 1, methotrexate was also given in combination with 5-FU. The chemotherapy regimen of patient 5 was not known.

The histopathological diagnosis of the biopsied specimens or the operated materials was poorly differentiated adenocarcinoma in all 6 patients from whom an appropriate sample was available. Signet ring cells were recognized in every patient.

Chromosome analysis The chromosomes were examined in cancerous effusions; pleural effusion in 1 patient and peritoneal effeusion in 6 (Table I). The cytological finding was Class V, adenocarcinoma in all the samples. The cancerous effusions were withdrawn with a heparinized syringe and the cells were enriched by centrifugation. Buffy coat was cultured in RPMI 1640 with 15% fetal

bovine serum for 2 to 24 h at 5% CO_2. Colcemid (0.1 μg/ml) was added 2 h before harvesting the cells. The chromosome preparations were made according to the ordinary air-drying method and G-banding was done in every case. Several chromosome slides were stained simply with Giemsa. Banded and non-banded metaphase spreads (54 to 258 cells) were photographed and the chromosome number was counted on the negatives to obtain the mode and the distribution of chromosome number. Sixteen to 42 selected metaphases were fully karyotyped according to the chromosome identification and karyotype designation proposed by the ISCN.[12]

RESULTS AND DISCUSSION

The modal chromosome number was hyperdiploid (49, 52, 52, 53) in 4 patients, hypertriploid (80) in 1, and hypotetraploid (85) in 1 (Table I). In patient 5, the cells with 46 chromosomes had a normal diploid chromosome complement, and the polyploid cells (106–231 chromosomes) did not provide a consistent karyotype, although there were many numerical and structural anomalies.[13] There was a considerable cell-to-cell variation of the karyotypes, but the modal karyotypes were obtained from the other 6 patients (Table II, Figs. 1–6).

Thus, in our 7 patients, the modal chromosome number varied from the diploid range to the tetraploid range; 4 patients were hyperdiploid (2n+), 1 hypertriploid (3n+), and 1 hypotetraploid (4n−). In 1 patient, the modal chromosome number was 46 in 58 of 62 cells.[13] There have so far been 15 reported patients with gastric cancer examined by banding methods[4, 6, 7, 14–16]; 7 analyses were done on the primary tissues,[6, 7] and 8 on the cancerous effusions.[4, 6, 14–16] Among these patients, the modality was distributed from hypodiploid (2n−) to hypertriploid (3n+); 2n− in 2 patients, 2n+ in 8, 3n− in 2, 3n in 1, and 3n+ in 2. The results are similar to ours. However, the modal chromosome number may be a little greater in cancerous effusions than in primary tumors, taking account of previous investigations in which banding chromosome analysis was not done[17–19]; 56% of the cases were in the diploid range and 44% triploid in the primary lesions versus 40% diploid, 38% triploid, and 22% tetraploid in the cancerous effusions. These results suggest that several additional or secondary chromosomal changes are added in the cells of cancerous effusion.

Among our 6 patients in whom the modal karyotype was obtained, several consistent and recurrent numeical alterations were detected; a gain of chromosome 7 in 5 patients, chromosome 13 in 4, and chromosomes 2 and 20 in 3 patients. Gains of chromosomes 7 and 8 were frequently observed among the 15 patients reported previously,[4, 6, 7, 14–16] each in 6 patients. Overall, gains of chromosomes 7 and 8 are the most frequent numerical changes in stomach cancer, observed in 11 and 8 out of 21 patients, respectively. In addition, an isochromosome for the long arm of chromosome 8, i(8q), was reported in 2 patients, previously.[6, 7]

In contrast, a loss of the whole chromosome was not frequent except for the Y chromosome.[7] In 2 of our patients, there was no normal X chromosome, but a part of the X chromosome was retained, and was involved in the structural rearrangements.

Table I. Summary of Gastric Cancer Patients in whom the Chromosomes were Studied in Cancerous Effusions

Patient		Age/Sex	Diagnosis		Operation	Survival (months)	Chromosomes				
No.	Name		Macroscopic	Histology			Material[c]	No. of cells examined		Mode	Range
1.	A.H.	48/F	Borr. 4	por, sig[a]	not operated	18	ascites[d]	54[e]	16[f]	49	46–101
2.	K.K.	41/M	Borr. 3	sig[a]	not operated	18	ascites	96	16	52	40–164
3.	R.M.	47/F	Borr. 4	por, sig[a, b]	total gastrectomy	16	ascites	79	42	52	42–96
4.	H.K.	38/F	Borr. 4	N/A	non-resectable (laparotomy)	20	pleural effusion	123	31	80	42–136
5.	M.K.	43/M	IIc+III	por, sig[a, b]	total gastrectomy	50	ascites	62	20	46	42–231
6.	T.A.	34/F	IIc+III	por, sig[a, b]	total gastrectomy	28	ascites	157	22	53	46–105
7.	T.H.	56/F	Borr. 4	por, sig[a, b]	non-resectable (laparotomy)	12+	ascites	258	28	85	42–199

a, b) Histological diagnosis was made according to the General Rules for the Gastric Cancer Study (11th Ed.)[11] *a*) on biopsied specimens, or *b*) operated materials. Abbreviations: por, poorly differentiated adenocarcinoma; sig, signet-ring cell carcinoma; N/A, not applicable because repeated biopsies revealed no malignant cells in the mucosa.
c) All the samples were Class V, adenocarcinoma, cytologically.
d) The patient had received intraperitoneal administration of cisplatin (100 mg) 6 months before the chromosome study.
e, f) Number of cells *e*) photographed and *f*) fully karyotyped.

Table II. The Modal Karyotype Obtained from Seven Patients with Gastric Cancer

Patient 1. 49,XX,+7,+11,−16*,−17*,−18*,ins(1)(q42q25q32)#,del(6)(q15),+der(8)t(8;?)(p11;?)*, del(9)(q22),+del(9),del(12)(p13),+der(17)t(17;?)(p?11;?)#;ins(1) or der(1)t(1;?)(q42;?) (Fig. 1)

Patient 2. 52,XY,+2*,+5,+6,+8*,+18,+18*,+20*,der(1)t(1;?)(p36;?)*,der(9)t(9;?)(p24;?) (Fig. 2)

Patient 3. 52,−X,−X,−3*,+7,+8,−10,+13,+13,+16,+20,+22,+der(X)t(X;?)(p22.1;?),inv(2)(q31q35), +der(3)t[del(3)(q23q27);10](p25;q11.2),der(4)t(4;?)(q31;?),del(5)(q22q31 or q13q15),der(6)t(6;?)(q25;?), +der(10)t(3;10)(p25;q11.2),del(11)(q14q25),ins(12)(q13;?) (Fig. 3)

Patient 4. 46,XX/80,−X,−X,+2,+2,+3*,+4,+5,+6,+7,+9,+9,+10,+10,+10*,−11,+13,+13*,+15, +19*,+20,+20,+20,+der(X)t(X;?)(q13;?),+der(X),+del(1)(p22p36.1),+del(3)(p12p25),+del(3), +del(7)(p13),+der(8)t(6;8)(p21.1;q21.3),+der(8)t(6;8),+der(11)t(X;11)(q13;p11.2),+der(11)t(X;11), +der(11)t(11;?)(q23;?),+del(12)(p12p12),+del(12),+der(17)t(17;?)(p11;?),+der(17),+mar1(C-size, submetacentric),+mar1,+mar2(D-size, metacentric),+mar2 (Fig. 4)

Patient 5. 46,XY/karyotype of polyploid cells, unidentifiable

Patient 6. 53,XX,+2,+7,+13,−18,+del(3)(q21q27)#,+der(9)t(9;13)(p22;q14),+der(18)t(18;?)(q23;?), +mar1(D-size, metacentric),+mar2(G-size, metacentric)#;del(3) or der(3)t(3;?)(q21;?) (Fig. 5)

Patient 7. 85,XX,+X,+X,+1,+2,+7,+7,+8,+8,+8*,+9*,+10,+10,+11,+11,+11*,+12,+13,+13,+13, +14,+15,+15,+16,+17,+18,+18,+19,+19,+20,+21,+22,+22*,t(3;5)(q21;q31),+(3)(p21HSR), +del(3)(q21q23),+dup(4)(q13→q31),+der(5)t(3;5)(q21;q31),+der(5),+del(6)(q21q27), t(7;10)(p22;q25),+i(17q),+mar1(A-size, submetacentric),+mar2(C-size, submetacentric),+mar3(G-size, metacentric),+1−42dmin (Fig. 6)

* Chromosome abnormalities observed in 40–80% of the cells examined.

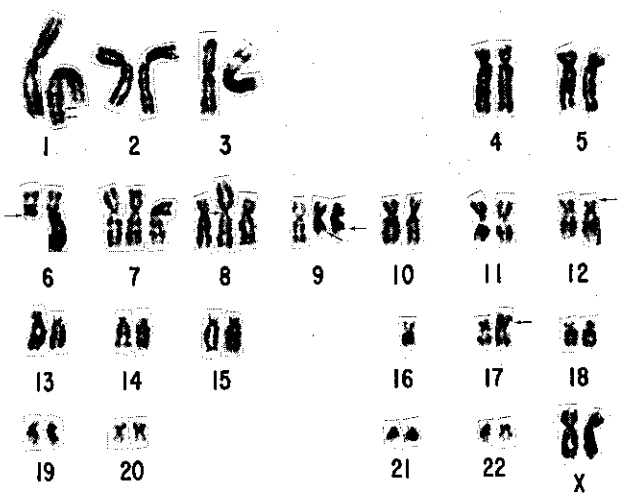

Fig. 1. A representative G-banded karyotype in patient 1. Arrows indicate rearranged chromosomes at the breakpoints. Trisomy 11 is not seen in this cell.

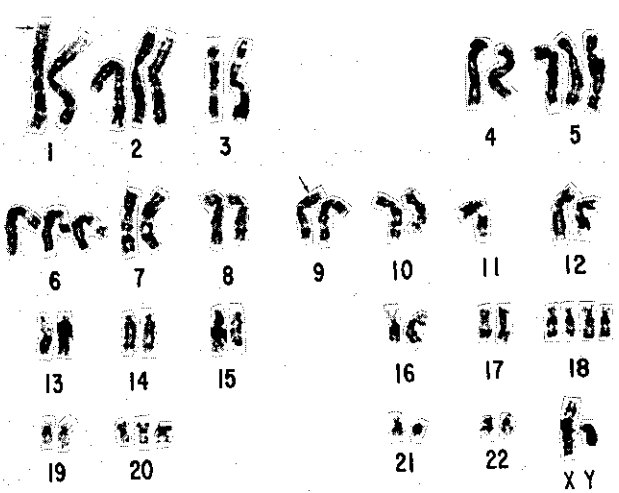

Fig. 2. A representative karyotype in patient 2. Arrows indicate rearranged chromosomes at the breakpoints.

Besides these numerical changes, structural rearrangements were also frequently encountered in our 6 patients. Chromosome 3 was recurrently involved in the short arm or in the long arm. Along the short arm, p25 band was involved in 2 patients and a homogeneously staining region (HSR), or an abnormally banded region (ABR), was seen at band p21 in another patient. Among 5 patients reported by Ochi *et al.*,[7] 4 structural rearrangements involving the short arm of chromosome 3 (band 3p21) were observed in 2 patients. The involvement of

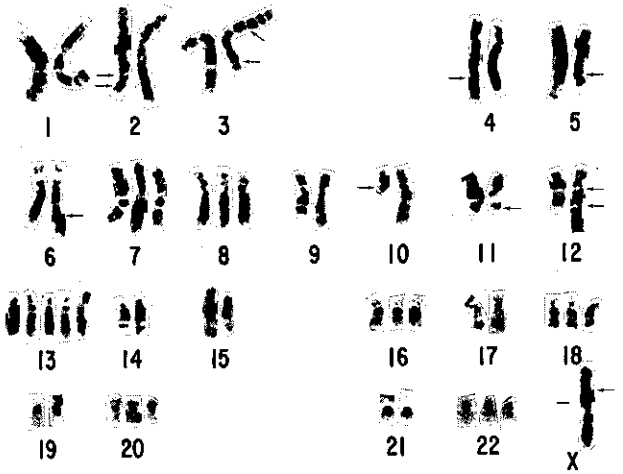

Fig. 3. A representative karyotype in patient 3. Arrows indicate rearranged chromosomes at the breakpoints.

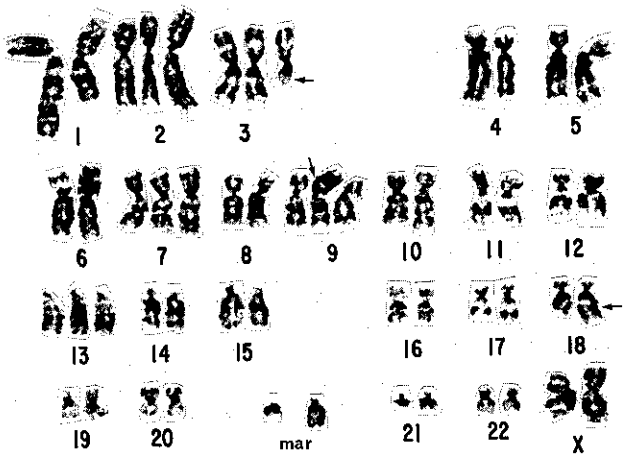

Fig. 5. A representative karyotype in patient 6. Arrows indicate rearranged chromosomes at the breakpoints. Rearrangement of the long arm of chromosome 1 is seen only in this karyotype.

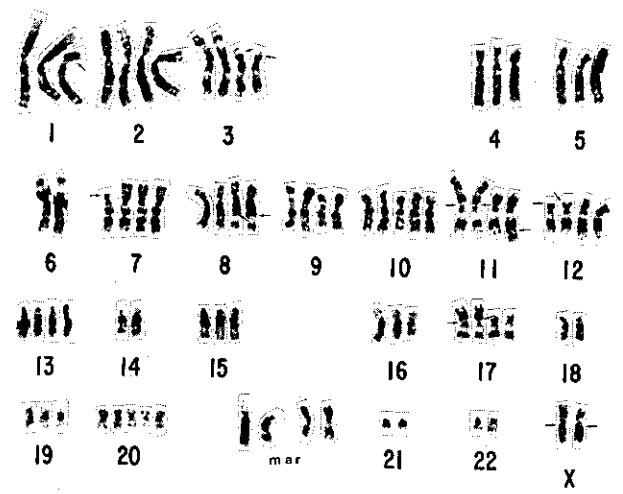

Fig. 4. A representative karyotype in patient 4. Arrows indicate rearranged chromosomes at the breakpoints.

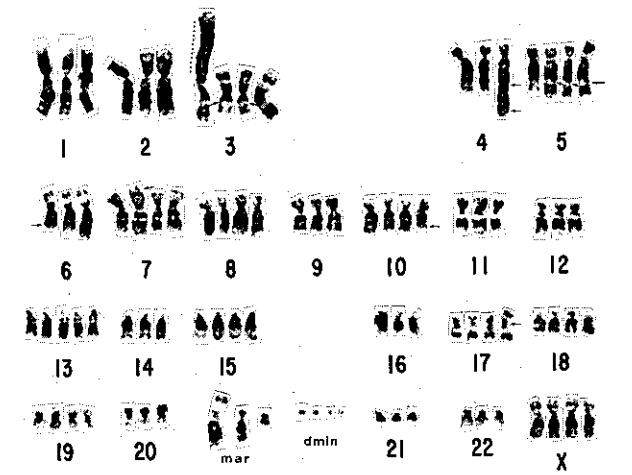

Fig. 6. A representative karyotype in patient 7. Arrows indicate rearranged chromosomes at the breakpoints. Dots represent an HSR on chromosome 3.

the short arm of chromosome 3 has been recognized in several other solid tumors, small cell lung carcinoma, renal cell carcinoma, pleomorphic adenoma, and ovarian carcinoma, each with a specific structural rearrangement.[1, 2)]

Interstitial deletions of the long arm of chromosome 3 were observed in 3 of our patients, in which a band q23 is commonly deleted. A complex rearrangement involving the 3q23 band was reported in 1 patient by Ochi *et al.*[7)] Thus, the band 3q23 may be a specific site for adenocarcinoma of the stomach. Reciprocal translocations involving a band 3q21 were detected in one of our patients, t(3;5)(q21;q31), and in another by Ochi *et al.*, t(3;12)(q21;p13).[7)] The band 3q21 is one of the chromosome bands recurrently involved in gastric cancer.

A 5q− and structural aberrations involving the long arm of chromosome 5 were seen in 2 of our patients. Two other patients were reported to have aberrations in the long arm of chromosome 5.[7, 15)] Thus, chromosome 5 anomalies are also one of the changes frequently observed, in which a band 5q31 seems to be preferentially involved (3 patients).

Another chromosomal region recurrently involved is a small segment including the centromere of chromosome

17. An isochromosome for the long arm, i(17q), was seen in 3 patients including 1 of our patients,[7,16] and unbalanced translocations in 2 of our patients and in 2 other patients reported by Ochi *et al.*[7]

Allelic deletions at specific chromosomal loci were studied in gastric cancer by molecular genetic approaches using polymorphic DNA markers.[8-10] Among chromosomal regions examined, loss of chromosomal heterozygosity was observed in the long arms of chromosomes 1, 5, 12, and 13. Structural aberrations in the long arm of chromosome 5 were detected by chromosome analysis as described above, but few cases have been observed to have the loss of a chromosome segment from the long arm of chromosome 1, 12, or 13 cytogenetically.

Although clinical and histopathological features were not described precisely in individual reported patients, there seem to be several recurrent and common chromosomal alterations. Some of these aberrations may be specific chromosomal defects leading to the initiation or progression of the stomach cancer.

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