

SHORT COMMUNICATION

CHROMOSOMAL CHANGES IN ADVANCED PRIMARY GASTRIC CANCER

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ABSTRACT

Successful cytogenetic analysis was performed in three advanced and one incipient case of gastric adenocarcinoma. Two of the advanced ones corresponded to undifferentiated adenocarcinoma, one of them with a chromosome number in the range of hyperdiploidy and the other in the hypodiploidy range. The third one was differentiated with an hypotriploid modal number. The incipient tumor (undifferentiated and tubular) presented a chromosome number in the range of diploidy.

Loss of chromosome 17 was detected in all three advanced gastric adenocarcinomas.

Structural alterations were observed at 1p in two tumors of differing histological types. A deletion was seen at 3p21 in one tumor and homogeneously staining regions (hsr) at 20p13 in another.

INTRODUCTION

That karyotypic alterations significantly influence the biological behavior of cancer cells is demonstrated by cytogenetic analysis in hematologic diseases as well as by consistent association of characteristic chromosomal changes with defined subtypes of these diseases (Sandberg, 1980; Sandberg *et al.*, 1988). Although the data are strongly suggestive of the role of karyotypic changes in neoplasia, only 1% of the published cytogenetic results deal with primary epithelial tumors, which represent 80% of all human cancers (Teyssier, 1989).

Gastric cancer is a common malignancy with marked geographic distribution in the world with Japan and Chile having the highest mortality rates (Armijo *et al.*, 1981; Correa, 1985). Even though little is known about the genetic changes which may be associated with the development of gastric adenocarcinoma (Wada *et al.*,

1988; Hirohashi and Sugimura, 1991), findings of amplification of a single base mutation of some oncogenes have been reported (Hattori *et al.*, 1990). Information from cytogenetic studies is limited and no consistent chromosomal abnormalities have been identified in primary gastric cancer (Ochi *et al.*, 1984, 1986; Casartelli and Rogatto, 1986; Ferti-Passantonopoulou *et al.*, 1987; Ito *et al.*, 1989; Tzeng *et al.*, 1991).

We report herein numerical and structural aberrations in primary gastric adenocarcinoma, according to the histological classification of Nakamura (1982).

MATERIAL AND METHODS

Specimens

Three advanced primary gastric tumors and an incipient one were obtained at surgery and histologically classified.

Cytogenetic analysis from short-term cultures

Cell cultures were obtained by tissue disaggregation with collagenase digestion (Limon *et al.*, 1986) or by explant (Aranda and Panasevich, 1975) in HAM F10 or MEM media, supplemented with FCS (10%)

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(Gibco). Cells in exponential growth were harvested *in situ*: after 22 hours in fresh growth medium, demecolcine (0.04 µg/ml) was added for four hours. Hypotonic treatment was performed with Hanks 5% in water pH 7. Fixation and GTG-banding were performed according to conventional methods (Dutrilleaux and Couturier, 1981).

Direct cytogenetic analysis

Tumor cells were mechanically disaggregated and resuspended for two hours in RPMI 1640 medium supplemented with FCS (10%) and demecolcine. Hypotonic treatment, fixation and GTG-banding were performed according to conventional methods. Chromosomal changes were described according to ISCN (1985, 1991).

RESULTS

Abnormal karyotypes were found in all three advanced gastric adenocarcinomas and in the incipient one. Of the advanced tumors, two corresponded to the histological classification of undifferentiated adenocarcinoma and one to the differentiated type. All of them had clonal chromosome abnormalities; a total of 13 structural anomalies were identified (Table I).

Numerical aberrations

Detailed chromosomal data are presented in Table II. Case 3 had a diploid chromosome modal number. The undifferentiated carcinomas had different ploidies: case 1

Table I - Clinical, histopathologic and cytogenetic features in four patients with gastric cancer.

Case	Age/Sex	Histopathologic data	Composite/karyotype
1	57/M	Undifferentiated	Hyperdiploidy 38-55,XY + X,i(1p),i(1q), + der(2)t(2;8)(p1;p13),del(3)(p21),ins(3;?)p21;?,+5,+8,+9,+9,+11,add(13)(q14),-17[cp4]
2	79/M	Undifferentiated	Hypodiploidy 36-41,XY,-6,-7,-12,t(15;15)(q26;q21)[2],-17,-19[cp5]
3	48/M	Mixed undifferentiated and tubular*	Diploidy 45-47,XY,+21[2]
4	60/M	Differentiated	Hypotriploidy 68-72,XXY,i(1q),del(1)(p22)x2,+4,+8,ins(9;?)p13;?,-10,+13,+add(13)(q13),+14,-15,-16,-17,-17,der(19)add(19)(p12),hsr(20)(p13)x2,+mar1x3[cp10]

*Incipient tumor

Table II - Distribution of chromosome counts.

Case	(Code)	Chromosome range	Hyperhaploid 24-34 (%)	Hypodiploid 35-45 (%)	Hyperdiploid 47-57 (%)	Hypotriploid 58-68 (%)	Hypertriploid 70-80 (%)	Mode	No. of mode (%)	Appar normal	Total
1	(TA13)	25-55	2 (15)	3 (23)	6 (46)	0	0	43	3 (23)	2	13
2	(TA22)	36-46	0	11 (73)	0	0	0	41	6 (40)	4	15
3	(TA20)	25-47	4 (25)	4 (25)	2 (13)	0	0	46	6 (37)	6	16
4	(TA15)	24-76	2 (13)	0	2 (13)	5 (33)	6 (40)	68	5 (33)	0	15

Chromosome number

Case	24	25	27	33	35	36	38	39	40	41	44	45	46	47	50	52	53	55	56	68	70	72	76	
1		2					1		2				2		1	1	1	3						
2						1		3	1	6			4											
3		2	1	1	1				1	1	1	6	2											
4	1	1																	2	5	1	4	1	

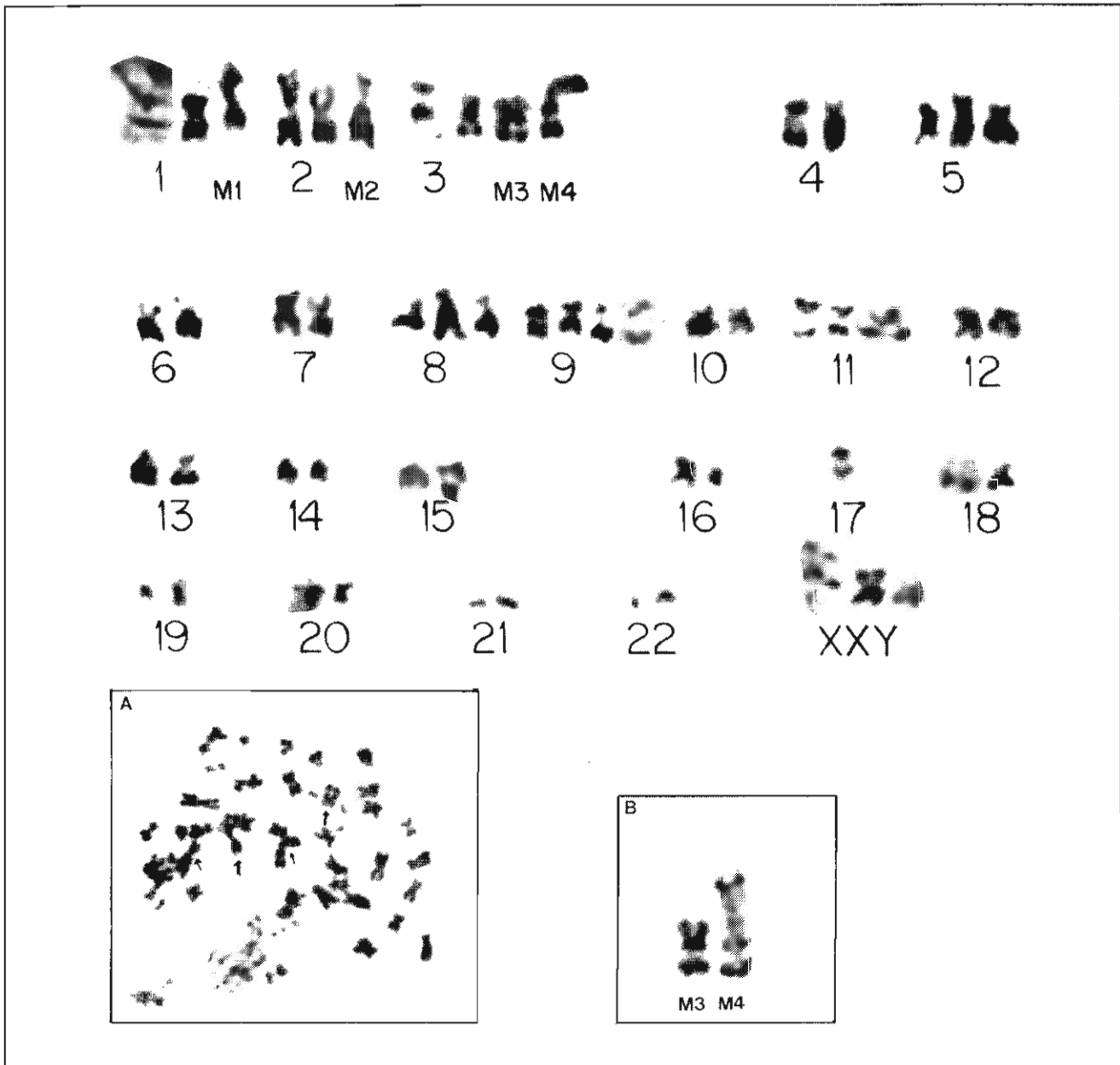


Figure 1 - Karyotype of Case 1: undifferentiated adenocarcinoma 47,XY,i(1p), +der(2)t(2;8) (p1;p13),del(3)(p21),+ins(3;?) (p21;?),+5,+8,+9,der(13)t(13;?)(q14;?)-14,-17,-17,-18[2]. Bottom insert: A:GTG banding metaphase. B: Partial karyotype from a different metaphase of case 1 showing M3: del(3)(p21) and M4: ins(3;?)(p21;?).

was hyperdiploid and case 2 was hypodiploid (Figure 1). Hypotriploidy was detected in case 4 (Figure 2).

Clonal loss of chromosome 17 was observed in all three cases of the advanced gastric cancers.

Structural aberrations

Structural alterations in 1p22 → 1pter were observed in case 4 and i(1p) in case 1. (Table I; M2 in Figure 2). In one undifferentiated type there was a duplicated marker with a deletion at 3p21 (M3 shown in Figure 1),

and another marker with additional material of unknown origin inserted in 3p21 (M4 in Figure 1). In the differentiated type, an hsr was observed in 20p13 (M5 in Figure 3).

DISCUSSION

Cytogenetic analysis of the four primary gastric cancers demonstrated different chromosome modal numbers for differentiated and undifferentiated types: a near-triploid karyotype in the former and a near-diploidy

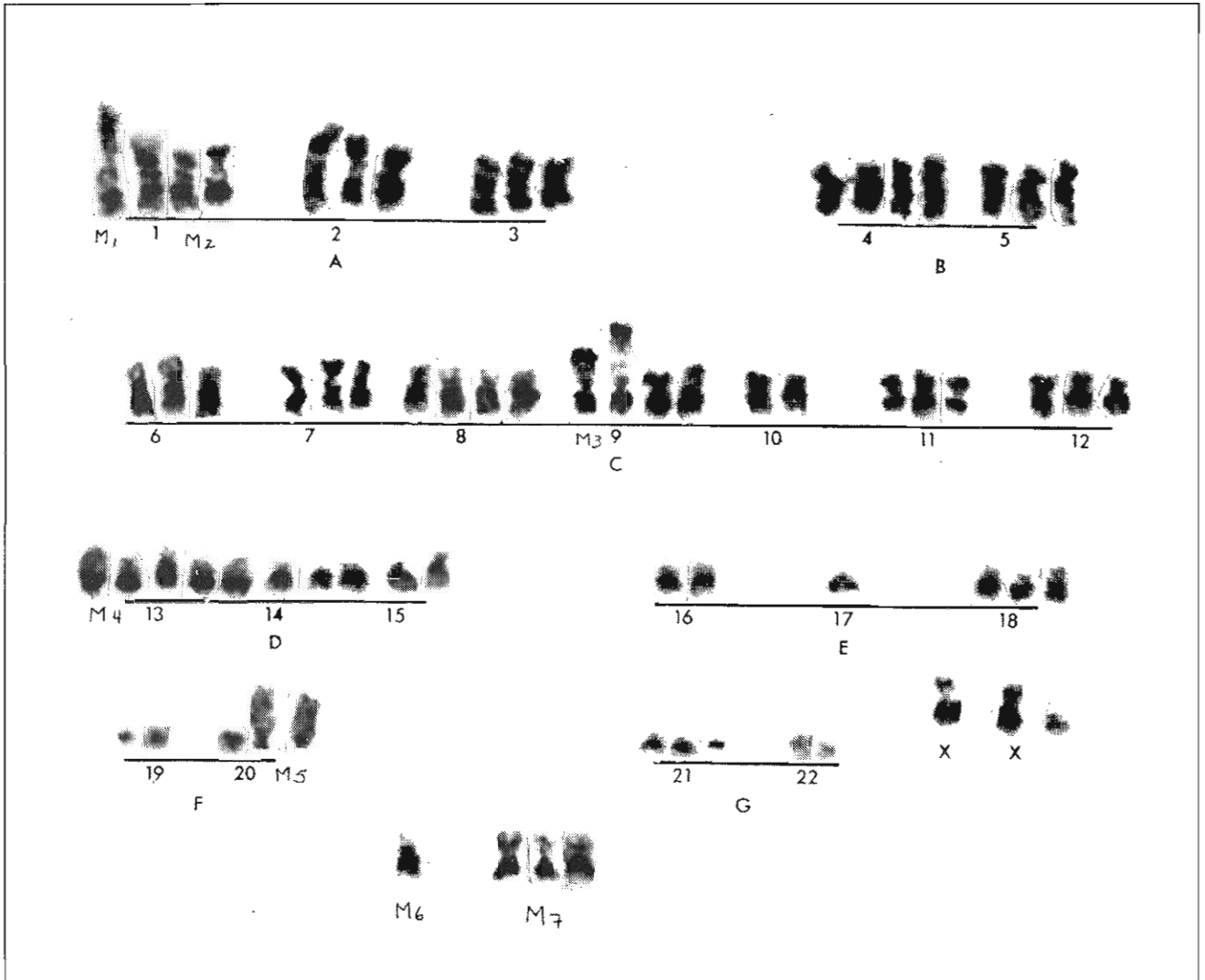


Figure 2 - Karyotype of Case 4: differentiated adenocarcinoma. 72,XXY,del(1)(p22)x2, +i(1q),+4,+8,+ins(9;?)(p13:?)x2,-10,+13,+add(13)(q13),-15,-16,-17,-17,der(19)t(19)t(19;?)(p12;?),hsr(20)(p13)x2,-22,+mar1x3.

in the latter. The two subtypes showed clonal structural alterations in 1p; the differentiated case showed a deletion in 1p22 → 1pter and in an undifferentiated one, i(1p) was found. This region involves 1p31.1 and 1p13, where the N-ras oncogene is located, which is most frequently involved in human malignancies (Davis *et al.*, 1984). A breakpoint detected in chromosome 3p21 (marker M3, Figure 1) has been reported by Ochi *et al.* (1986), as a frequent alteration in gastric cancer and has been also observed in nearly 100% of small-cell lung carcinomas (Yokota *et al.*, 1987).

The inserted material of unknown origin observed in chromosome 3 (M4, Figure 1) and chromosome 9 (M3, Figure 2 and Figure 3) and the hsr in 20p13, could involve a gene amplification process in gastric cancer. This same explanation has been proposed by Casartelli and Rogatto (1986) in relation to their finding of "Micromarkers". Nevertheless the observations of hsr in 3p21, 9p13 and 20p13 in the differentiated types of other advanced gastric

cancers (Ochi *et al.*, 1986; Ferti-Passantonopoulou *et al.*, 1987), do not demonstrate that this corresponds to a gene amplification process.

Clonal loss of chromosome 17 was observed in the two subtypes of gastric carcinoma. These results suggest that chromosome 17 could play an important role in the multistep process of carcinogenesis.

The total number of studied cases by cytogenetic analysis is still not sufficient and further investigations are needed to clarify whether certain specific chromosomal changes are really involved in development of gastric cancer, or if they relate to some histological subtype.

RESUMO

Análises citogenéticas foram realizadas com sucesso em três casos de adenocarcinoma gástrico avançado e um incipiente. Dois casos avançados corresponderam a adenocarcinomas indiferenciados, um com número cromossômico na faixa hiperdiplóide e outro na faixa

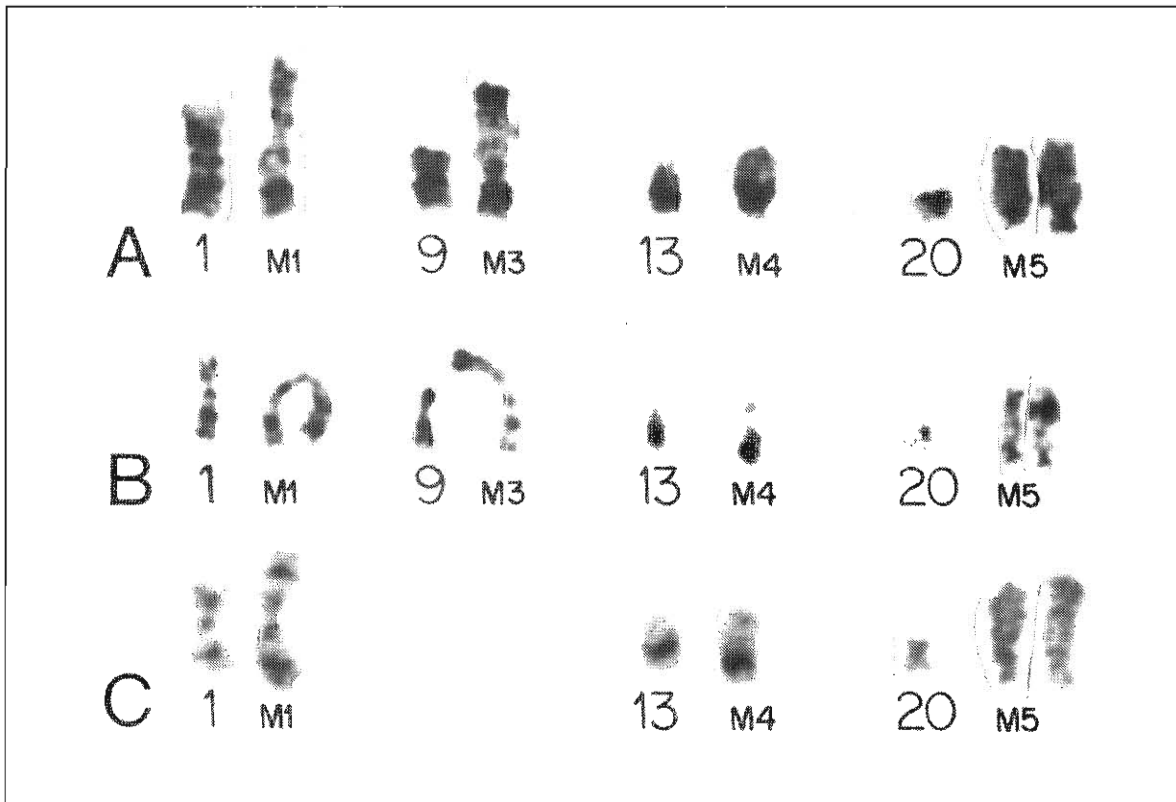


Figure 3 - (A, B and C): Partial karyotypes of case 4 showing $i(1q)$ (M1), $ins(9;?)(p13;?)$ (M3), $add(13)(q13)$ (M4), and $hsr(20)(p13)$ (M5).

hipodiplóide. O terceiro foi diferenciado com um número modal hipotriplóide. O tumor incipiente (não diferenciado e tubular) apresentou um número cromossômico na faixa da diploidia.

A perda do cromossomo 17 foi detectada nos três casos de adenocarcinoma gástrico avançado.

Alterações estruturais foram observadas em 1p em dois tumores de tipo histologicamente diferentes. Uma deleção - 3p21 - foi observada em um tumor e regiões homogeneamente coradas em 20p13 em outro caso.

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