



Cytogenetic Analysis of Barrett's Mucosa and Adenocarcinoma of the Distal Esophagus and Cardia

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ABSTRACT: We performed flow cytometry and cytogenetic analysis of 37 adenocarcinomas of the distal esophagus and cardia, of which 22 arose in Barrett's mucosa. Two of eight analyzed specimens of Barrett's mucosa had clonal chromosomal abnormalities. In 19 cases clonal chromosomal abnormalities were found in tumor tissue. The complex pattern of cytogenetic changes did not differ among the adenocarcinomas arisen in Barrett's esophagus, and those in the distal esophagus without Barrett's mucosa or cardia. Abnormal karyotypes with multiple and complex rearrangements were seen in 11 cases and with single or a few numeric changes in eight. Losses of chromosomes 4, 18, 21, and Y were the most frequent numeric changes. Loss of the Y chromosome was observed in eight of 26 tumors of males (31%). Gains of chromosomes 14 and 20 were also frequent numeric changes. Structural abnormalities were observed in 13 of the abnormal karyotypes (68%). The chromosome arms most frequently rearranged were 1p, 3q, 11p and 22p. The chromosome arm most frequently contributing to losses was 1p, with the shortest region of overlap being 1p22-33. The chromosome arms most often involved in gains were 11p and 22p, and i(3q) was the isochromosome that was most frequently identified.

INTRODUCTION

There has been a striking increase in the incidence of adenocarcinoma of the esophagus and the esophagogastric junction in North America and Western Europe in recent years [1, 2]. Most esophageal and gastroesophageal adenocarcinomas appear to arise from Barrett's esophagus [3]. As a consequence of persistent damage, the squamous epithelial lining of the esophagus is replaced by columnar epithelium. The risk of developing an adenocarcinoma in Barrett's esophagus is at least 30-125 times that expected for a similar population without Barrett's esophagus [4]. Barrett's adenocarcinoma occurs as a result of progression of severe dysplastic changes in the abnormal mucosal lining of the esophagus, involving a series of genetic alterations in the cells that comprise the Barrett's epithelium [5]. A better understanding of the underlying mechanisms of carcinogenesis in Barrett's esophagus and the discovery of markers identifying the subset of patients at high risk would be of great importance. The correlation between

dysplastic changes and aneuploidy has been demonstrated by flow cytometric (FCM) techniques [5, 6]. A few studies demonstrated clonal chromosome abnormalities in Barrett's epithelium and adenocarcinoma [7-10]. The karyotypes were often complex, but loss of the Y chromosome was a frequent finding. Genetic changes affecting 3q and the 11p13-15 region have been described in esophageal adenocarcinomas as well as in gastric cancer [8, 9]. However, since the cytogenetic study of solid tumors and pre-malignant tissues is difficult, there are no large series reported.

We report cytogenetic studies of 37 adenocarcinomas of the distal esophagus and cardia of which the majority (59%) arose in Barrett's mucosa. In these cases ($n = 22$) cytogenetic analysis was also performed on Barrett's epithelium.

MATERIALS AND METHODS

Patients

Patients with an esophageal carcinoma were referred to the Rotterdam Esophageal Tumor Study Group for evaluation and treatment. Three groups were investigated: 1) patients with an adenocarcinoma in Barrett's esophagus; 2) patients with an adenocarcinoma in the distal esophagus without Barrett's mucosa; and 3) patients with an adenocarcinoma of the cardia. All patients underwent an esophagectomy without preoperative radiotherapy or che-

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motherapy, and were included in the study during the period November 1989 to August 1992. Tissue specimens of 37 patients (26 male and 11 female; mean age of 62 years) were analyzed by histopathologic, FCM, and cytogenetic methods.

Pathology

Based on histopathologic findings and site of the tumor, the 37 adenocarcinomas of the patients were divided into three subgroups:

1. Adenocarcinoma in Barrett's esophagus, if the bulk of the tumor was located in the distal esophagus and columnar epithelium was found proximal to the tumor at microscopic examination ($n = 22$).
2. Adenocarcinoma in the distal esophagus, if the bulk of the tumor was located in the distal esophagus and no columnar epithelium could be demonstrated proximal to the tumor by microscopic examination ($n = 10$).
3. Adenocarcinoma of the cardia, if the bulk of the tumor was located in the cardia with tumor growth into the distal esophagus ($n = 5$).

From all 37 resection specimens fresh tissue samples of the esophageal mucosa and the tumor were taken out and divided into three parts. One part of every tissue sample was used for histopathologic analysis, one for cytogenetic analysis, and a third part was stored in liquid nitrogen (-70°C) for FCM. In a few cases tissue samples were taken from lymph node metastases.

If columnar epithelium was found, specimens were designated as no dysplasia, mild-, moderate-, and severe dysplasia according to criteria for epithelial dysplasia in the stomach as described by Morson et al. [11]. In all tumor specimens adenocarcinoma was found and the differentiation grade of the tumor was determined.

Table 1 Clinical and histopathologic characteristics of 22 patients with an adenocarcinoma in Barrett's esophagus

Patient no.	Sex	Age	Adenocarcinoma ^a		Mucosa ^a	
			pTNM	Grade ^b	Type ^c	Grade of dysplasia
1	F	76	T3N1M0	G1		
3	M	61	T1N0M0	G3		
5	M	58	T3N1M0	G2	Squamous	
9	M	45	T2N0M0	G3	Barrett	Mild
11	M	63	T1N0M0	G3	Barrett	Mild
12	F	65	T3N1M0	G3		
13	M	59	T3N1M0	G3	Squamous	
17	M	80	T1N0M0	G2	Barrett	Moderate
18	M	67	T3N1M0	G3		
19	M	69	T3N1M0	G3 ^d	Barrett	No
20	F	67	T3N1M1	G3	Barrett	Moderate
23	F	77	T3N0M0	G2	Barrett	No
24	F	68	T1N0M0	G2	Barrett	Severe
25	F	75	T1N0M0	G2	Barrett	Mild
26	F	67	T3N1M1	G3		
27	M	77	T3N1M0	G3	Barrett	No
29	M	70	T2N0M0	G2	Barrett	Mild
31	M	53	T3N1M0	G2	Squamous	
32	F	64	T2N0M0	G1	Barrett	No
36	F	73	T3N1M1	G2	Barrett	Moderate
38	M	50	T3N1M0	G3		
39	M	70	T3N0M0	G3		

^aHistopathologic diagnosis of tissue samples for flow cytometric and cytogenetic analysis.

^bDifferentiation grade: G1 = well differentiated; G2 = moderately differentiated; G3 = poorly differentiated.

^cHistologic diagnosis of mucosa specimens on which cytogenetic and flow cytometric analysis were performed.

^dTissue sample from a lymph node metastasis.

Table 2 Flow cytometric and cytogenetic analysis of Barrett's mucosa

Patient no.	Flowcytometry (DNA indices)	Number of metaphases analyzed				Karyotype of clonal abnormality
		Total	Normal	Abnormal nonclonal	Abnormal clonal	
9	Aneuploid (1.93, 3.43)					
11	Diploid	1		1		
17	Diploid	16	7	4	5	45(34-46),X,-Y[cp5]
19	Diploid	26	20	6		
20	Tetraploid	6	4	2		
23	Diploid	2	2			
24	Aneuploid (1.18, 2.32)	3	2	1		
25	Aneuploid (1.61)	5			1 + 4 ^a	72<3n>,XXX,+3,-4,-5,-5,-6,+8,+add(9)(p22),+del(10)(q23),-11,+12,-14,+add(15)(p12),+add(15)(q21),+add(17)(p12),-18,+add(19)(q13),+20,-21,+22,+mar1,+mar2[cp4]
27	Diploid	1	1			
29	Diploid					
32	Diploid					
36	Diploid					

^aIndicates metaphases that were counted, with markers identified, but which could not be completely karyotyped.

FCM

Fresh tissue samples for FCM were immediately deep-frozen and stored in liquid nitrogen (-70°). These specimens were prepared for FCM according to the method described in Vindelov et al. [12]. The nuclear suspension was filtered through a 40- μ m mesh filter and stained with pro-

pidium iodide (Sigma Chemical Co., St. Louis, MO). Cellular DNA content of at least 10,000 cells was measured on FACS scan (Beckton Dickinson, Mountain View, CA). The data were fed into a Data General computer. Aneuploidy was determined both by visual inspection of the histograms and confirmation of an aneuploid population

Table 3 Flowcytometric and cytogenetic analysis of adenocarcinoma in Barrett's esophagus

Patient no.	Flowcytometry (DNA indices)	No. of metaphases analyzed				Karyotype of clonal abnormality
		Total	Normal	Abnormal nonclonal	Abnormal clonal	
1		10			6 + 4 ^a	39-42<2n>,X,-X,+3,-4,add(10)(q26),+hsr(11)(p14),-13,-17,-18,-20,-21,add(22)(p11),+mar,+dmin[cp10]
3		1	1			
5		14	9	2	1 + 2 ^a	80<4n>,XXX,-Y,-Y,del(1)(p21p34),+add(1)(p22),+inv(2)(q13q33),-3,-4,-5,-6,del(7)(q31),-7,-9,-9,-10,-10,-10,+11,add(12)(p12),i(13)(q10),-13,-13,-13,-14 add(15)(q26)x2,add(18)(p11),-18,-18,-21,-21,-21,add(22)(p12)x2,+r,+mar1x2,+mar2,+mar3
9	Aneuploid (1.85, 3.29)	9	6	3		
11	Tetraploid	6	4	2		
12	Aneuploid (1.65)	20	16	2	2	46-47,XX,+2mar[cp2]
13	Diploid	28	3		8 + 7 ^a	37-42<2n>,X,i(Y)(q10),del(1)(p21p34),+add(3)(q27),-5,i(7)(p10),add(8)(q24),-9,-11,-12,-13 i(13)(q10),-15,-19,-20,-22,add(22)(q13),+r,+mar[cp15]
					7 + 3 ^a	69(64-83)<3n>,XX,i(Y)(q10),+2,+3,+add(3)(q27),+4,-5,+add(6)(q26)x2,i(7)(p10),+i(7)(p10),add(8)(q24)x2,-10,-11,-12,i(13)(q10)x2,+14,+15,-17,-18,-19,-20,+21,add(22)(q13)x2,+rx2,+mar[cp10]
17	Aneuploid (1.56)	34	12	6	16	45(34-45),X,-Y[10],-10,-14,-17,-18,-21[cp16]
18	Aneuploid (1.49)	37	17	2	18	45(36-47),X,-Y[9],-2,-6,-7,-9,-11,-18,-19,-22[cp18]
19	Aneuploid (1.50, 2.05)	11	5	2	4	66-69<3n>,X,-Y,add(9)(p22),+mar[cp4]
20	Diploid	8	6	2		
23	Aneuploid (1.98, 2.93)	12	9	3		
24	Aneuploid (1.20, 2.43)	6	4	2		
25	Aneuploid (1.61)	6			4 + 2 ^a	63-71<3n>,XXX,+X,del(1)(p21p33),+3,-4,-5,+8,add(9)(p22),+10,-13,+14,add(15)(p12),add(15)(q21),-18,+add(19)(q13),add(21)(p21),+22,+mar1,+mar2[cp6]
26	Aneuploid (1.47, 1.78)	4	4			
27	Aneuploid (1.49, 2.07)					
29	Aneuploid (1.69)	8	6	2		
31	Aneuploid (1.42)	7	1	2	2 + 2 ^a	51-55<2n>,X,add(Y)(p13),+del(1)(p22),+del(2)(p15),+add(3)(p26),+del(3)(q24),+9,+11,+add(14)(q32),hsr(14)(p11),i(17)(p10)x2,add(18)(p11),+20,+20,-21,-22,-22,+mar[cp4]
32	Aneuploid (1.99, 2.79)	3	2	1		
36	Aneuploid (1.72)	8			5 + 3 ^a	76(61-95)<3n>,XX,-X,+1,+del(1)(p22),+del(2)(p13),+der(2;3)(q10;q10),+del(3)(q21),-4,add(4)(q2?),+der(4;12)(q10;q10),add(6)(p22),del(7)(q31)x2,-8,der(9)t(9;10)(q34;q22)x2,+hsr(11)(p14),-12,+13,der(15)t(1;15)(p22;p13)x3,-17,+20,+20,+20,+r,+mar1,+mar2,+mar3[cp8]
38		1	1			
39		23	10	8	5	39-45<2n>,XY,-19,-21[cp5]

^aIndicates metaphases that were counted, with markers identified, but which could not be completely karyotyped.

Table 4 Clinical and histopathologic characteristics of patients with an adenocarcinoma in the distal esophagus without Barrett's mucosa (DO) or cardia (C)

Patient no.	Sex	Age	Adenocarcinoma		
			pTNM	Grade ^a	Localization
2	M	44	T3N1M1	G3	DO
6	M	68	T3N1M0	G2	DO
7	M	46		G2 ^b	DO
8	M	44		G2 ^b	DO
10	F	62	T3N1M0	G3	DO
16	M	63	T2N1M1	G3	DO
28	M	44	T2N0M0	G2	DO
34	M	54	T3N1M0	G2	DO
35	M	68	T3N1M0	G3	DO
37	M	53	T2N1M0	G3	DO
14	M	69	T3N2M0	G3	C
21	M	64	T3N1M0	G3	C
22	M	41	T3N1M0	G3	C
30	M	63	T3N2M1	G3	C
33	F	73	T3N2M0	G2	C

^aDifferentiation grade: G1 = well differentiated; G2 = moderately differentiated; G3 = poorly differentiated.

^bTissue sample from a lymph node metastasis.

by calculating the deoxyribonucleic acid index as defined by the convention of nomenclature for deoxyribonucleic acid [13]. A diploid histogram contained a G0/G1 peak and a G2/tetraploid peak above DNA index 1.90 and below DNA index 2.10, with a G2/tetraploid fraction up to 10%. G2/tetraploid fractions above 10% were considered abnormal. An aneuploid population of cells was defined as a G0/G1 population of cells that produced a discrete peak separate from the diploid population, constituting $\geq 5\%$ of the cells in the biopsy specimens. The coefficient of variation was $\leq 6\%$ in all cases.

Cytogenetics

Fresh tissue samples of esophageal mucosa and adenocarcinoma obtained from the resection specimens were minced with sharp scissors into pieces approximately ≤ 1 mm in diameter. The pieces were incubated in Dulbecco's modified Eagles' medium (DMEM)/Ham's F12 culture medium containing 5 $\mu\text{g}/\text{mL}$ Amphotericin B, 100 IU/mL penicillin, and 0.2 mg/mL streptomycin for 1 h. The suspension was washed in medium, then medium containing collagenase II (200 IU/mL) was added for several hours to obtain enzymatical disaggregation of cells. The suspension was washed two times in medium and dispersed into 50-mL cell culture T-flasks with 10 mL DMEM/Ham's F12 culture medium with 10% fetal calf serum, 0.3 mg/mL glutamine, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin. The cultures were maintained at 37°C in a humidified atmo-

Table 5 Flow cytometric and cytogenetic analysis of adenocarcinoma in the distal esophagus without Barrett's mucosa

Patient no.	Flow cytometry (DNA indices)	No. of metaphases analyzed				Karyotype of clonal abnormality
		Total	Normal	Abnormal nonclonal	Abnormal clonal	
2		1	1			
6	Aneuploid (1.53)	5	2		3	45(45-64)<2n>,X,-Y[cp3]
7	Aneuploid (1.65)	21			12 + 9 ^a	63-68<3n>,XXYY,+der(1)t(1;9)(p12;q12),+der(1)t(1;9)(p12;q12),+2,i(3)(q10)x2,add(3)(p26),add(4)(q23),-1,-12,der(12)t(10;13)(q23;p12)x2,del(13)(q12),+del(13)(q12),+del(13)(q12),+14,+15,der(16)t(13;16)(q12,p13)+der(16)t(13;16)(q12;p13),-17,-18,-19,-20,add(21)(q21)x2,-21,del(22)(p11)x2,der(22)t(3;22)(p12,p13),+der(22)t(3;22)(p12;p13),+r,+mar[cp21]
8	Diploid	17	10	2	5	91-94<4n>,XXYY,+12,+18[cp5]
10	Aneuploid (1.56, 1.94)	23	2		5 + 16 ^a	50-64<2n>,XX,add(1)(p33),+add(1)(q43),+2,del(3)(p14),+i(3)(q10),del(4)(p12),-4,add(5)(q35),+5,add(6)(p23),del(7)(q31),+der(7;11)(p10;q10),del(8)(p21),del(11)(p14),add(11)(p15),+hsr(12)(p12),add(13)(p11),-13,i(14)(q10),add(15)(q26),+16,del(17)(q25),+add(17)(p12),+20,+20,+21,+21,add(22)(p12),+mar[cp21]
16	Aneuploid (1.69)	32	19	2	11	40(28-45)<2n>,X,-Y[5],-1,-2,-4,-5,-9,-11,-13,-17,-18,-22[cp11]
28	Aneuploid (2.17)	16	12	1	3	44-45,X,-Y[cp3]
34		8			4 + 4 ^a	58-60<3n>,XX,-Y,add(1)(p11),del(3)(p24),+del(3)(p24),-4,i(5)(p10),-6,add(7)(p1?),add(8)(q24)x2,add(9)(p21),-9,hsr(11)(p14),del(12)(q21),-12,i(14)(q10),-14,-15,-17,-17,-18,-18,-18,-21,-21,+r,+4mar[cp8]
35	Aneuploid (1.73)	1	1			
37	Diploid	1	1			

^aIndicates metaphases that were counted, with markers identified, but which could not be completely karyotyped.

Table 6 Flow cytometric and cytogenetic analysis of adenocarcinoma in the cardia

Patient no.	Flow cytometry (DNA indices)	No. of metaphases analyzed			Karyotype of clonal abnormality
		Total	Normal	Abnormal nonclonal clonal	
14		6	6		
21	Tetraploid	1	1		
22	Aneuploid (1.41, 2.69)	1	1		
30		6	5	1	
33		11	2		9
					60-77<3n>,XX,-X,-1,add(3)(q29),+i(3)(q10),-4,-5,del(6)(q24)x2,+add(6)(p22),add(8)(p22),-12,add(13)(q34),+add(13)(q34),add(14)(q32)x2,-14,add(15)(p13),-15,+16,+16,+16,+16.add(17)(q25),del(17)(q22),-18,add(20)(p11),-20,-21,-22,+mar[cp9]

sphere of 5% carbon dioxide in air. The specimens were harvested for cytogenetic analysis after 2 or 3 days of growth *in vitro* (short-term culture). To synchronize the cells in the cell cycles the cultures were treated with methotrexate (1 µg/mL) overnight and thymidine (10⁻⁵ M) 6 h before harvesting [14]. Metaphase cells were arrested by exposure to colcemid (0.1 µg/mL) for 1 h. The mitotic cells were loosened by shaking the culture flasks, washed with medium, swelled in KCl 0.075 M at 37°C for 8 min, and fixed according to standard procedures using methanol:acetic acid (3:1). The remaining monolayer was flooded with KCl-EGTA for 20 min and scraped out with a rubber policeman, washed with KCl 0.075 M, and processed as above. The chromosomes were identified using RFA and QFQ banding. The quality of metaphases was irregular, and not all cells could always be fully karyotyped. Karyotype designations were in accordance with the International System for Human Cytogenetic Nomenclature [15]. As a control, preoperative blood samples of the patients were obtained and cytogenetic analysis of the leucocytes was performed.

RESULTS

Clinical Characteristics

Table 1 shows the clinical and histopathologic characteristics of 22 patients with an adenocarcinoma in Barrett's esophagus. The histologic diagnosis of the mucosal specimens on which cytogenetic and FCM analysis were performed are listed according to the mucosal type (i.e., Barrett's epithelium or squamous epithelium). In one patient (no. 19), tumor tissue for cytogenetic analysis was obtained from a lymph node metastasis, while the tissue culture of the primary tumor was not successful.

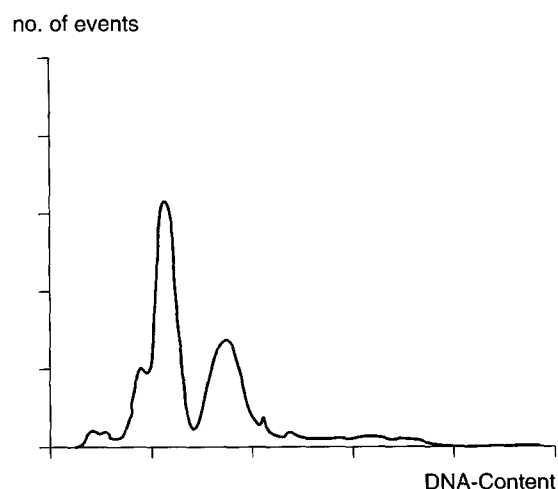
Barrett's Mucosa

The results of flow cytometric (12 cases) and cytogenetic analysis (eight cases) of Barrett's mucosa are listed in Table 2. FCM showed aneuploidy in three cases, of which clonal abnormalities were found in one (no. 25). In this patient the DNA index of Barrett's mucosa was 1.61, correlating with the modal chromosome number (near triploid) found at cytogenetic analysis. One of the diploid specimens of Barrett's mucosa showed a hypodiploid karyotype

with loss of the Y chromosome. The number of metaphases was insufficient in four cases (fewer than five mitoses). In three patients the mucosal tissue samples that were analyzed cytogenetically contained only squamous epithelium. In these cases Barrett's mucosa seen histologically in their resection specimens was not present in the tissue samples for cytogenetic analysis. These samples all showed normal karyotypes. In 11 patients of 22, tissue cultures of the mucosa were not successful, which prevented cytogenetic analysis.

Adenocarcinoma

Table 3 lists the data of flow cytometric and cytogenetic analysis of 22 adenocarcinomas in Barrett's esophagus. FCM showed aneuploidy in 14 cases, of which the DNA indices varied between 1.42 and 1.72 in 10 cases. Four of these 10 tumors also showed near-triploid karyotypes (nos. 19, 25, 31, and 36). Patient 13 exhibited two distinct clonal populations, one hypodiploid and another near triploid, both showing the same markers. Of the 12 cytogenetic abnormal clones identified, five were hypodiploid and two of these corresponded to near-triploid FCM indices, a situation similar to that of patient 13. There were no

Figure 1 Histogram of flow cytometric analysis of tumor tissue of patient no. 7.

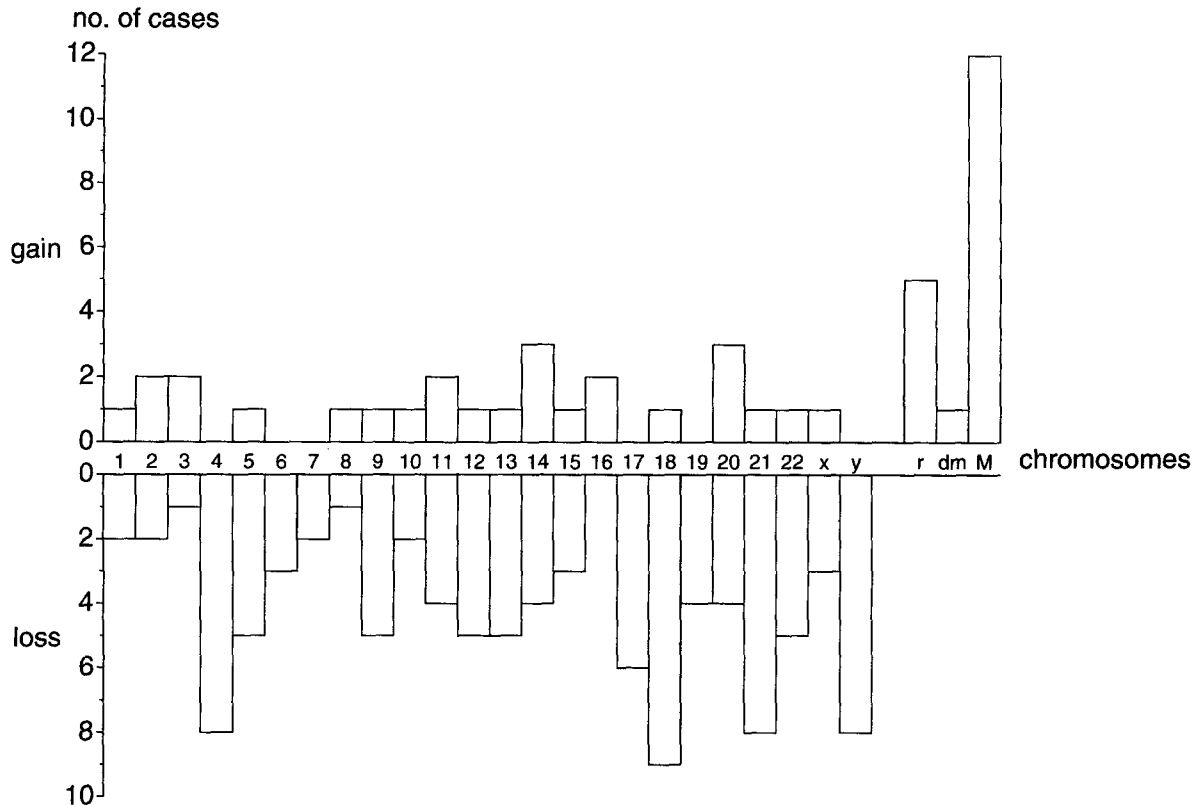


Figure 2 Histogram of all clonal numerical chromosome changes observed in tumor cells of 19 patients with an adenocarcinoma in Barrett's esophagus (11), the distal esophagus without Barrett's mucosa (7), and cardia (1). Gains and losses depicted are determined relative to ploidy. Unidentified markers are also shown. r = ring chromosome, dm = double minute, mar = marker chromosome.

mitoses found in one case, and an inadequate number of metaphases in four cases. In four tumors aneuploidy or tetraploidy was found by FCM, but this could not be confirmed cytogenetically.

Table 4 shows the clinical and histopathologic characteristics of the patients with an adenocarcinoma in the distal esophagus without Barrett's mucosa ($n = 10$) and cardia ($n = 5$). In two patients the tumor appeared to be irresectable at laparotomy and tumor tissue was obtained from lymph node metastases (nos. 7 and 8). All esophageal mucosa specimens were of the squamous cell type. Cytogenetic analysis of squamous epithelium was performed in six cases, and at random, numeric changes were found in four of them. In the other nine patients tissue cultures of the squamous epithelium were not successful.

The results of FCM and cytogenetic analysis of adenocarcinoma located in the distal esophagus without Barrett's mucosa and cardia are listed in Tables 5 and 6, respectively. FCM showed aneuploidy in six of the 10 adenocarcinomas of the distal esophagus, of which the DNA index correlated with the modal chromosome number found at cytogenetic analysis in two (nos. 7 and 10) (Table 5). An FCM histogram of patient 7 showed a hypodiploid shoulder and an aneuploid peak with a DNA index of 1.65 (Fig. 1). Furthermore, three others had DNA indices varying between 1.53 and 2.17, but hypodiploid cytogenetic

clones were found (nos. 6, 16, and 28). An inadequate number of metaphases was found in three cases. FCM showed a tetraploid population in one case and aneuploidy in another case of adenocarcinoma of the cardia (Table 6). In both cases only one metaphase was analyzed and showed a normal karyotype. More than 10 metaphases were analyzed in one case, in which an abnormal clone was found. Constitutional chromosomal abnormalities were not found in any of the patients by cytogenetic analysis of peripheral blood lymphocytes.

Clonal chromosomal abnormalities in Barrett's mucosa were seen in two cases with near-diploid and near-triploid chromosome numbers, respectively. In 19 cases clonal chromosomal abnormalities were found in tumor tissue. These were analyzed for the presence of common numeric and structural aberrations. All chromosomes contributed to numeric changes. Gains and losses of chromosomes, determined relatively to ploidy, were plotted on a histogram (Fig. 2). When compared with the ploidy of individual tumors, whole chromosome losses were more common than gains. Losses of chromosomes 4, 18, 21, and Y were the most frequent numeric losses. Loss of the Y was observed in eight of 13 tumors of males (62%). Chromosomes 14 and 20 were most frequently gained. Loss of chromosome 16 was not observed. Structural abnormalities were observed in 13 of the abnormal clonal karyotypes (68%). All

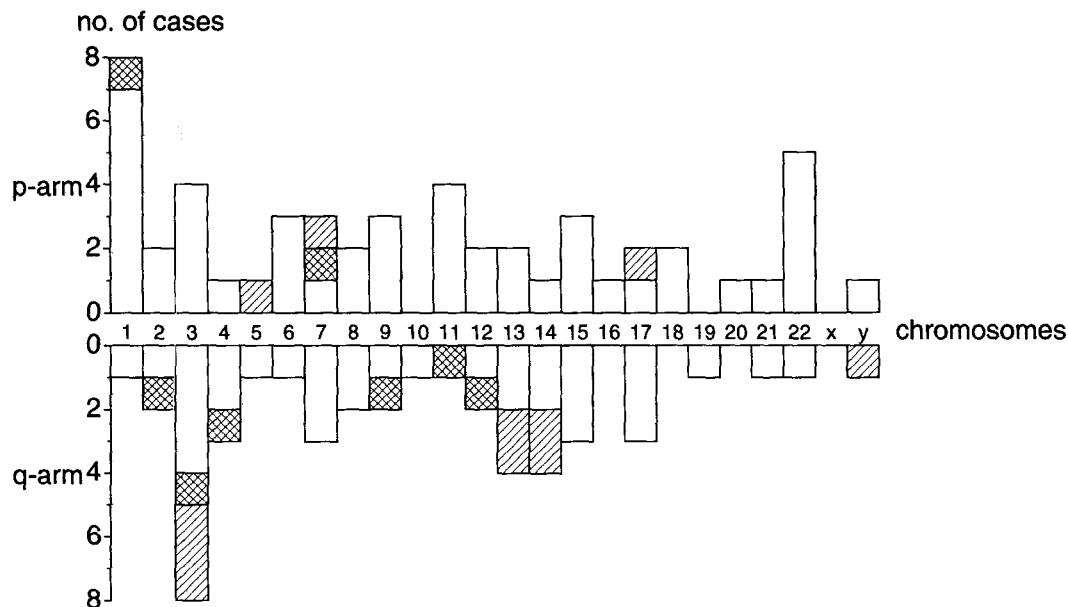


Figure 3 Histogram of all clonal structural chromosome changes observed in tumor cells of 12 patients with an adenocarcinoma in Barrett's esophagus (8), the distal esophagus without Barrett's mucosa (3), and cardia (1). Diagonal bars = isochromosomes; hatched bars, unbalanced translocations).

chromosome arms except 10p, 16q, 18q, 19p, 20q, and chromosome X participated in structural changes (Fig. 3). The chromosome arms most frequently rearranged were 1p, 3q, 11p, and 22p. Abnormalities of chromosome 1p arm frequently resulted in (partial) deletion, with the shortest region of overlap at 1p22-33 (Fig. 4). In contrast, chromosome 11p and 22p rearrangements often showed gain of material. A homogenous staining region (hsr) of 11p14 was observed in three cases and gain at 11p15 in one case, whereas a deletion on chromosome arm 11p was seen in one case with the break point at 11p14 (Fig. 4). Structural rearrangements of chromosome arm 3q were frequently observed, including deletions in two cases, gains in two cases, an unbalanced translocation in one case, and isochromosomes in three cases (Fig. 4). The breakpoint occurred at the centromere in four cases. Several recurrent isochromosomes were identified. The most frequent were i(3q) in three cases, i(13q) in two cases, and i(14q) in two cases.

DISCUSSION

In 1976 Nowell hypothesized that cancer develops in association with an acquired genetic instability that predisposes to the acquisition of abnormal clones of cells with accumulated genetic errors [16]. It has been demonstrated that neoplastic progression in Barrett's esophagus is associated with a similar process of clonal evolution [5, 6, 10, 17]. In our study we analyzed 37 adenocarcinomas of the esophagus and cardia, of which 22 arose in Barrett's esophagus. In Barrett's mucosa we found normal karyotypes, except in one case where a complex karyotype was found with the same markers and rearrangements as in the

adenocarcinoma of the same patient. A theoretical possibility in this case is that the abnormal tissue obtained from the Barrett's segment that was spatially separate from the cancer contained an unidentified microscopic focus of carcinoma. A specific karyotypic change common to all cases with genetic abnormalities was not found. The absence of differences in the complex pattern of cytogenetic changes between adenocarcinomas in Barrett's esophagus, the distal esophagus without Barrett's mucosa or cardia, suggests a common pathway of origin at all three anatomic sites. This is similar to the results of others [8, 9]. A good correlation between DNA indices of the aneuploid tumors and the modal chromosome numbers of the abnormal karyotypes was found in most of the tumors with multiple and complex chromosomal rearrangements. Recurrent combination of hypodiploid and triploid clones correspond to a pattern of clonal progression that is characterized by chromosome losses followed by secondary duplication and losses, a mechanism which has previously been described in adenocarcinoma [18]. Whereas balanced translocations were seldom found in this study, losses due to missing chromosomes or apparently unbalanced rearrangements (derivative chromosomes, deletions, and isochromosomes) were frequently observed. Marker chromosomes were common and some of the missing chromosomal material obviously resides in these unidentified chromosomes. Whole chromosome losses of 4, 18, and 21, and in males, Y, were the most frequent numeric changes in this series. Frequent numeric loss of chromosomes 4, 18, 21, and Y has been described in colorectal carcinoma [19-22]. Loss of the Y chromosome has been demonstrated in different gastrointestinal malignancies [19, 20, 23] and brain tumors [22, 24]. In Barrett's esophagus and esophageal adenocarcinoma loss of the Y chromosome also appears to be a

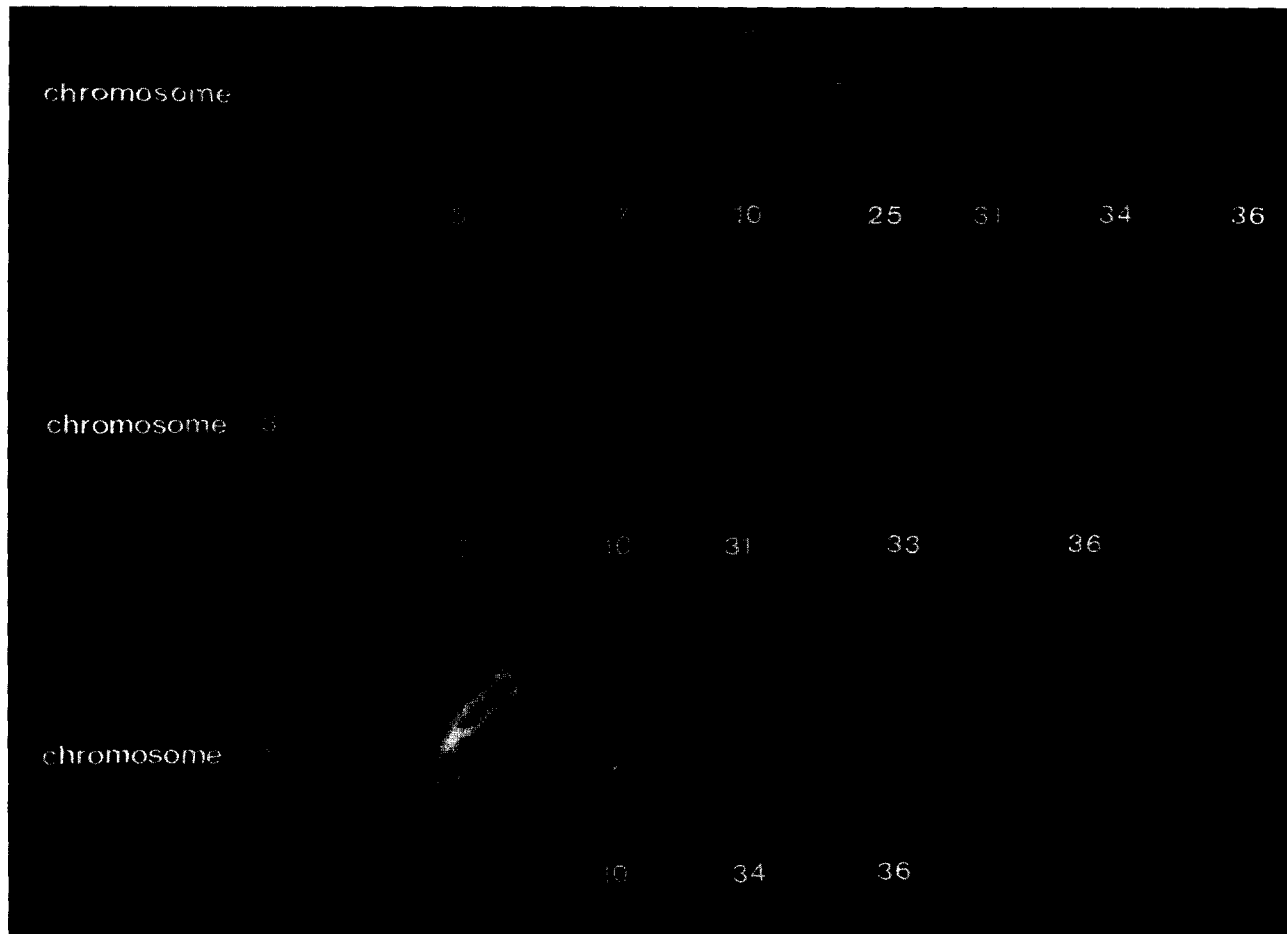


Figure 4 Examples of rearrangements affecting chromosome arms 1p, 3q, and 11p in the tumors studied. Patient numbers are indicated under the rearranged chromosomes. R bands are used in the majority of cases and Q bands for patient nos. 1 (chromosome 11), 5 (chromosome 1), 31 (chromosomes 1 and 3), and 36 (chromosome 3).

common occurrence [7, 8, 17, 25]. It has been suggested that tissues retaining rapid cellular proliferation rates in elderly men are more prone to loss of the Y chromosome [26]. In our series loss of the Y chromosome in Barrett's mucosa and adenocarcinoma occurred in 62% of the male patients, but not merely the oldest ones. Furthermore, cytogenetic analysis of peripheral blood lymphocytes did not reveal Y loss. Although there is a male predominance in the incidence of Barrett's esophagus and esophageal adenocarcinoma [1, 4, 27], the significance of Y loss is unclear, because there are no cancer genes linked to the Y chromosome so far. Recurrent gains of chromosomes 14 and 20 were seen in this series. Extra copies of chromosome 20 were also found in colorectal carcinomas [19, 22]. Structural abnormalities of chromosome arms 1p, 3q, 11p, and 22p were most frequently observed. Structural rearrangements of the short arm of chromosome 1 resulted most often in deletions, with the shortest region of overlap being 1p22–33. Frequent rearrangements of chromosome arm 1p were also described in gastric and colorectal carcinomas [20, 23]. *N-ras*, a transforming gene from a neuroblastoma cell line, has been assigned to 1p22–31 [28, 29].

Rao et al. described structural rearrangements of 3q in six of nine gastric and esophageal adenocarcinomas [9]. Allelotype analysis in osteosarcomas suggests the existence of tumor suppressor genes on the long arm of chromosome 3 [30]. Structural rearrangements in the 11p13–15 region were found in Barrett's adenocarcinomas and other adenocarcinomas of the esophagus and stomach [8]. Genetic changes affecting the 11p13–15 region have previously been associated with Wilm's tumor, hepatoblastoma, rhabdomyosarcoma, and carcinoma of the bladder and breast [31–33]. Furthermore, the *H-ras-1* protooncogene has been localized to band 11p15 [33]. Whether these cancer genes are implicated in the progression of oesophageal tumors should be investigated using molecular techniques.

These studies show that Barrett's mucosa and adenocarcinoma can be successfully karyotyped using banding techniques. A chromosomal abnormality common to all tumors was not identified, although hot spots for structural rearrangements have been seen in chromosomes 1p, 3q, 11p, and 22p, as well as frequent losses of chromosomes 4, 18, 21, and Y.

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