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Chromosomal analysis of Barrett's cells: demonstration of instability and detection of the metaplastic lineage involved

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Barrett's esophagus is lined by columnar and goblets cells with gastric and intestinal characteristics. Despite the association between goblet elements and malignancy, it was not demonstrated that other columnar cells lineages are not related to neoplasia. Chromosomal abnormalities were described in metaplasia adjacent to Barrett's neoplasia, but it is unknown which metaplastic lineages are involved. This work assessed the frequency and the type of chromosomal abnormalities in Barrett's esophagus without neoplasia and performed the identification of the metaplastic cells carrying chromosomal gains. Barrett's esophagus biopsies were collected and processed for short-term cell culture and cytogenetic analysis. Combined immunofluorescence/fluorescence *in situ* hybridization was performed in cases exhibiting chromosomal gains by using antisera against intestinal (MUC2) and gastric (MUC5AC and MUC6) apomucins and chromosome pericentromeric alpha satellite DNA probes for the chromosomes involved. Each case was scored for the number of spots (0, 1, 2, >2) in 200 nonoverlapping nuclei. Columnar and goblet cells were separately assessed. Short-term cell cultures were achieved in 40/60 cases (67%). There were clonal abnormalities in 27/40 cases (68%) and tetraploid (4n) clones in 10/40 (25%). Structural alterations were detected in 14/40 (35%) with recurrent breakpoints at 1q21, 15q15 and 15q22. Numerical changes (trisomies 7 and 18 and loss of Y) occurred in 16/40 (40%). Gains of chromosomes 7 and 18 were more frequent in columnar than in goblet cells (9.8% *vs* 0.7% ($P<0.05$)) and (7.9 *vs* 1.9% ($P<0.05$)) respectively. These alterations were detected in cells exhibiting gastric as well as intestinal features and were more frequent in cells without apomucin production. Conclusions: (1) chromosomal instability is a common finding in Barrett's esophagus without neoplasia. (2) The two metaplastic populations are committed, chromosomal gains being more frequent in columnar nongoblet than in goblet cells. (3) The two metaplastic phenotypes, gastric and intestinal, are equally involved.
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Barrett's esophagus is a premalignant condition in which the normal stratified squamous epithelium is replaced by a metaplastic columnar lining with intestinal differentiation.[1,2] It develops in 10–14% of patients with long-standing gastroesophageal reflux disease and it is the sole known precursor of esophageal adenocarcinoma,[1,2] the tumor whose incidence increased over the last decades at a rate exceeding that of any other cancer type.[3–5]

During the process of metaplasia, a heterogeneous admixture of cellular elements with distinct phenotypes replaces the normal squamous esophageal epithelium.[1,2] This metaplastic lining is a complex structure including intermingled gastric and intestinal-type epithelia.[6–9] The latter, is identified by the presence of goblet-shaped cells, the metaplastic element regarded as the hallmark of Barrett's esophagus due to its association with cancer risk.[9,10] Nevertheless, the prevalent cellular elements of Barrett's esophagus are the columnar nongoblet cells, which exhibit gastric and intestinal characteristics as goblet cells do.[11]

Gastric and intestinal mucosas are protected by a mucus layer of high molecular weight glycoproteins synthesized by normal epithelial cells in a cell- and tissue-specific pattern.[12,13] MUC5AC and MUC6 are the protein cores of gastric mucus, foveolar and mucopeptic, respectively. MUC2 is the protein

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constituent of the intestinal mucus. This cell- and tissue-specific organization makes these apomucins reliable markers of gastric and intestinal phenotypes.

It is widely accepted that the development of Barrett's adenocarcinoma follows a stepwise mechanism from metaplasia to dysplasia and carcinoma.[10] This pathway to malignancy is the morphologic counterpart of a progressive accumulation of genetic events (gross chromosomal and/or subtle DNA sequence abnormalities) that lead to genomic instability.[14–17] Aneuploidy and increased G2/tetraploid fraction, signs of gross DNA chromosomal abnormalities, have been related to Barrett's tumorigenesis.[16]

Conventional cytogenetics is a useful tool to characterize chromosomal abnormalities and a previous study demonstrated the presence of chromosomal defects in Barrett's esophagus.[17] However, there are very few reports using this technique[14,17] and, to our knowledge there are no studies correlating the distinct columnar population of Barrett's esophagus without evidence of neoplastic changes with the presence of genetic abnormalities.

The aims of this study were:

1. To assess the frequency and to characterize chromosomal abnormalities in Barrett's esophagus without dysplasia or cancer.
2. To identify the metaplastic cellular lineage carrying these changes.

Materials and methods

This study was performed in two distinct phases.

In Phase I, epithelial cells from samples of Barrett's esophagus without dysplasia or carcinoma were collected, processed for short-term cell culture and analyzed by cytogenetic analysis after assessment of its phenotype. In Phase II, we used a combined immunofluorescence/fluorescence *in situ* hybridization (IF-FISH) technique performed on formalin-fixed, paraffin-embedded tissue sections from biopsies obtained during the same endoscopy to identify cells carrying recurrent numerical chromosomal abnormalities and to characterize their morphology and immunophenotype.

Phase I

Biopsy samples collection

Endoscopic biopsies were obtained from patients enrolled in the Barrett's esophagus Surveillance Program of the Instituto Português de Oncologia de Lisboa, after informed consent. The diagnosis of Barrett's esophagus was based on the histological demonstration of intestinal metaplasia, recognized by the presence of goblet cells, in biopsies taken from red velvet mucosal segments of the distal esophagus.[18] Only patients with long segments ($\geq$3 cm) of Barrett's esophagus with biopsies negative for dysplasia or carcinoma were included. Presently, all the patients have at least 5 years of regular endoscopic follow-up after inclusion in this study and none developed dysplasia or cancer. Biopsies were collected according to Levine *et al*[19] protocol and samples of 2–3 extra biopsies were obtained for cytogenetic analysis. To prevent contamination, biopsies for cytogenetics were collected during endoscope insertion before gastric and duodenal observation. The material for tissue culture was placed in phosphate-buffered saline (PBS) medium containing penicillin, streptomycin and amphotericin B, and immediately processed. Biopsies for diagnostic purposes were routinely processed and subsequently used in Phase II studies.

Tissue culture

To establish short-term cell cultures, we used a modified Khan *et al*[20] technique. Briefly, the tissue was mechanically desegregated with scalpels and enzimatically digested using collagenase II (400 U/ml) in Hank's Balanced Salt Solution for 16 h at 37°C, until small cell clumps were obtained. The clumps were suspended and washed in RPMI medium and plated out in T25 Primaria flasks (Falcon) and T25 vitrogen-coated flasks (Nunc). The culture medium used for initiation has been used previously for culturing breast epithelial cells.[21] Cell cultures were then incubated at 37°C in a humidified incubator containing 5% carbon dioxide and left undisturbed for 5 days. Cultures were examined daily on inverted microscopy. The culture medium was replaced twice a week after initiation of cell growth. Upon reaching confluence, cells were trypsinized (Trypsin-EDTA; Gibco-BRL) and plated onto Lab-Tek® cover glass chamber slides used to perform cytogenetic analysis.

Phenotypic evaluation of the cells obtained by short-term culture

After the initial subculture, an aliquot of cells was grown on coverslips and fixed in 1% formaldehyde PBS 1× for 10 min. The epithelial phenotype of *in vitro* growing cells was immunohistochemically assessed by using monoclonal antibodies anti-cytokeratin (CAM 5.2 (Becton Dickinson 349205) and AE1/AE3 (Zymed 18-0132)). For the identification of gastric characteristics, foveolar and mucopeptic, two monoclonal antibodies against the apomucins MUC5AC and MUC6, CLH2 and CLH5 (courtesy of Leonor David, MD, PhD), respectively, were used. Intestinal differentiation was identified by using a monoclonal antibody for the apomucin MUC2 (NCL-MUC-2; Novocastra) normally present at the goblet intestinal cells.

Cytogenetic analysis

After 15–20 days of *in vitro* growth, metaphase cells were arrested by exposure to colcemid® (Gibco) for 6–16 h. The mitotic cells were swelled with NaCl

0.021% at room temperature for 35–45 min and fixed progressively with methanol:acetic acid (3:1). The chromosomes were G-banded according to standard cytogenetic methods, and chromosome abnormalities were considered as clonal according to the recommendations of the ISCN.[22] Briefly, clonality was defined by the presence of any of the following criteria: (1) two cells with the same structural abnormality or nonrandom gain of a given chromosome; (2) three cells with loss of a given chromosome.

Phase II

Combined IF-FISH study
Endoscopic biopsy samples from the patients with recurrent numerical chromosome abnormalities were used. Formalin-fixed and paraffin-embedded sections with 4 μm were processed by using a combined IF-FISH protocol developed for the simultaneous detection of the cellular immunophenotype and the numerical alterations detected previously. The phenotypic characterization of the metaplastic elements was performed with the same panel of antibodies used in Phase I for the identification of gastric and intestinal characteristics in short-term cell culture cells. The slides were incubated for MUC5AC and MUC6 for 24 h at 4°C and for MUC2 for 30 min at room temperature after: (1) antigen retrieval in citrate buffer (pH 6.0) using microwave oven for 20 min at 750 W; (2) pretreatment with $2 \times$ SSC for 30 min at 45°C; (3) proteolytic digestion with pepsin at 4 mg/ml in HCl 0.2 N (pH 2.0) for 2 min. The reaction was developed with avidin-Cy3 (Sigma, St Louis, MO, USA). Subsequently, FISH was performed on the same slides. According to the results of previous cytogenetic analysis, we used biotinylated probes specific for the pericentromeric regions of chromosome 7 (Zymed, San Francisco, CA, USA) and chromosome 18 (Q-BIOgene, llkirch, France). Probes were detected by anti-biotin FICT (Ventana, Tucson, AZ, USA). The nuclei were counterstained with DAPI (Vectashield Vector, Burlingame, CA, USA). IF-FISH analysis was performed with an epifluorescent microscope (Olympus BX40) equipped with a triple band beam splitter and emission filters *Dapi/Green/Orange* (Olympus BX51). Each case was scored for the number of hybridization spots (0, 1, 2 and >2) in 200 distinct, nonoverlapping, single or side-by-side, nuclei. Columnar nongoblet and goblet cells were separately assessed. Normal gastric and colon epithelia were used as controls for the IF expression of the gastric (MUC5AC and MUC6) and intestinal (MUC2) apomucins, respectively.

Statistical analysis
For statistical analysis χ^2 and Fisher's exact test were used. A value of $P<0.05$ was considered as significant.

Results

Phase I

The study included 50 patients, 39 male and 11 female, with a mean age of 61.2 years (ranging from 24- to 83-years old). Barrett's esophagus length ranged from 3 to 10 cm. Ten patients had two samples collected for short-term cell culture.

Establishment and Phenotypic Characterization of Short-Term Cell Cultures

A total of 60 biopsy samples were processed for short-term cell culture. Efficient growth of epithelial cells was obtained in 40 of the 60 (67%) cases. In 20 cases, short-term cell culture was not achieved because of microbial contamination ($n=15$) or insufficient growth ($n=5$). The epithelial lineage of the cultured cells was confirmed by positive staining for the cytokeratins CAM 5.2 and AE1/AE3. The presence of a mixed, intestinal and gastric phenotype characteristic of Barrett's esophagus was confirmed by the expression of MUC2, MUC5AC and MUC6.

Cytogenetic Analysis

The results of cytogenetic analysis are summarized in Table 1.

Cytogenetics aberrations were observed in 27 of the 40 (68%) successfully cultured cases. In 14 of them multiple abnormal clones were detected. Tetraploid (4n) cell populations were present in 10 of the 40 (25%) cases. Structural chromosomal abnormalities were detected in 14 of the 40 cases (35%) being duplications, and balanced translocations the most prevalent alterations. Chromosomes 1, 5, 9, 11, 14, 15 and 22 were involved in at least two cases. Breakpoint distribution analysis identified 1q21, 15q15 and 15q22 as recurrent breakpoints. Numerical chromosomal alterations were present in 16 of the 40 (40%) cases. Loss of chromosome Y was detected in 14 out of 27 (52%) male cases with efficient cell growth; gains of chromosomes 7 and 18 were present in six and two cases, respectively.

Phase II

Combined IF-FISH study
The results of the combined IF-FISH study are summarized in Tables 2, 3 and 4.

IF-FISH analysis for the phenotypic characterization of the elements carrying the abnormalities was performed in eight cases with chromosomal gains, six of chromosome 7 and two of chromosome 18. A total number of 4800 cells (4287 (89%) columnar and 513 (11%) goblet) was analyzed for the presence

Table 1 Cases with cytogenetics abnormalities

Case	*Sex*	*Age*	*Karyotype*
2	F	71	46,XX,t(7;15)(q11;q24)(cp2)/46,XX,del(17)(q11)(2)/45∼47,XX, +7(cp3)/92,XXXX(3)/46,XX, (9)
5	M	69	46,XY,add(14)(p11)(3)/46,XY (2)
6	M	58	46∼47,X,−Y,+7(cp2)/45,X,−Y (6)/46,XY (10)
9	F	47	46,XX,dup(11)(q13qter) (2)/92,XXXX (3)/46,XX (14)
10	M	58	46∼47,XY,1qh+c,+18(cp5)/92,XXYY,1qh+c (2)/46, XY,1qh+c (10)
10	M	58	46,XY1qh+c (13)
12	M	53	45,X,−Y,dup(5)(q13q31) (2)/47,XY,+18 (3)/46,xy (15)
13	M	72	45,X,−Y,22ph+c(11)/46,XY,22ph+c (12)
15	M	65	45,X,−Y (11)/46,xy (11)
17	M	46	46,X,−Y,add(11)(p15),+20 (2)/46,X,−Y,+20 (5)/47,XY,+7, (7)/41∼45,X,−Y,del(1)(p11) (3), +7 (3),cp (3)/92, XXYY (2)/46,XY (4)
18	M	54	47,XY,+7/2)/45,X,−Y (cp7)/92XXYY (2)/46,XY (8)
18	M	54	92,XXYY (3)/46,XY (10)
20	M	83	45,X,−Y (10)/46,XY (1)
22	M	72	46,XY,der(6)t(6;15)(p23;q22),der(15)t(6;15)(p23;q15)?del(q15q22) (2)/46,xy (7)
23	M	60	92,XXYY (5)/46,XY
24	M	77	46,XY,t(15;16)(q;p) (2)/92,XXYY,T(15;16)(q;p)x2 (5)/45,X,−Y (5)/46,X,−Y,+7 (5)/91,XXY,−Y (6)/92,XXYY (3)/46,XY (7)
26	M	78	43∼45,X,−Y (cp3)/46,XY (8)
27	M	76	41∼45,XY,t(8;9)(q13;q32) (cp8)/41∼45,X,−Y (cp7)/46,XY (11)
30	M	81	46,XY,t(1;5)(q…;q…),t(3;22)(p…;q…)/46,XY,t(1;5)(q…;q…)/46,XY
32	M	60	45,X,−Y (3)/46,XY (20)
34	M	77	46,XY,t(4;12)(q26∼27;q15∼21),inc (cp2)/46X,−Y,+9(6)/45,X,−Y(7)/46,XY (7)
38	F	31	91,XXYY,−19(4)/92,XXYY (1)/46,XY (21)
39	M	66	45∼46,X,−Y,+7 (cp4)/46,XY (19)
42	M	71	90,XX,−Y,−Y (5)/92,XXYY (2)/46,XY (13)
44	M	47	46,XY,tas(21;22)
47	F	79	46,XY,t(14;15)(q…;q…) (10)/46,XX
48	M	51	46,XY,dup(1)(q…),16qh+c (3)/46,XY
49	F	76	46,XX,t(9;9)

M, male; F, female.

Table 2 Number of cells analyzed in combined IF-FISH study

Cells analyzed	*Chromosome 7*	*Chromosome 18*
Total	**3600**	**1200**
Columnar cells	3188 (89%)	1099 (92%)
Goblet cells	412 (11%)	101 (8%)
No. of cells with gains	**318 (9%)**	**89 (4%)**
Columnar cells	315 (99%)	87 (98%)
Goblet cells	3 (1%)	2 (2%)

Total – number of cells analyzed for chromosomes 7 and 18.
Columnar cells – number (%) of columnar cells analyzed for chromosomes 7 and 18.
Goblet cells – number (%) of goblet cells analyzed for chromosomes 7 and 18.
No. of cells with gains – number of cells with gains of chromosomes 7 and 18.
Columnar cells – number (%) of columnar cells with gains of chromosomes 7 and 18.
Goblet cells – number (%) of goblet cells with gains of chromosomes 7 and 18.

of numerical alterations, on chromosomes 7 (3600) and 18 (1200).

Gain of chromosome 7 was detected in 318/3600 (9%) cells (315 (99%) columnar and 3 (1%) goblet). Gain of 7 was more frequent in columnar than in goblet elements (315/3188 (10%) *vs* 3/412 (1%) $P<0.05$). For the evaluation of the phenotypic characteristics of the cells in the six cases with gain of chromosome 7, we analyzed 1200 (200 per case) cells per IF-FISH assay (Table 3). In IF-FISH assay for MUC2, 1064 columnar and 136 goblet cells were evaluated. MUC2 was detected in 207/1064 (19%) columnar and in 91/136 (67%) goblet elements. In columnar cells, gain of chromosome 7 was more frequent in elements without MUC2 (87/857 (10%) *vs* 4/207 (2%) $P<0.05$). There was no difference between the frequency of chromosome 7 gain in goblet elements with and without MUC2 (2/45 (4%) *vs* 0/91). The IF-FISH assay for MUC5AC evaluated 1078 columnar and 122 goblet cells. MUC5AC was observed in 755/1078 (70%) columnar and in 73/122 (60%) goblet elements. There was no difference between the frequency of chromosome 7 gain in columnar cells with (Figure 1) and without MUC5AC (90/755 (12%) *vs* 42/323 (13%)). Gain of chromosome 7 was not observed in goblet cells (0/122) in IF-FISH assay for MUC5AC. IF-FISH assay for MUC6 analyzed 1046 columnar and 154 goblet cellular elements. MUC6 was detected in 429/1046 (41%) columnar and in 60/154 (39%) goblet cells. In columnar cells, gain of chromosome 7 was more frequent in elements without MUC6 (69/617 (11%) *vs* 23/429 (5%) $P<0.05$). There was no difference between the frequency of chromosome 7 gain in goblet elements with and without MUC6 (1/60 (2%) *vs* 0/94).

Gain of chromosome 18 was detected in 89/1200 (7%) cells (87 (98%) columnar and 2 (2%) goblet). Gain of 18 was more frequent in columnar than in goblet elements (87/1099 (8%) *vs* 2/101 (2%)

Table 3 Phenotypic characteristics of cells and chromosome 7 status

Chromosome 7	*Columnar*						*Goblet*					
	MUC2		*MUC5AC*		*MUC6*		*MUC2*		*MUC5AC*		*MUC6*	
	+ve	*−ve*	*+ve*	*−ve*	*+ve*	*−ve*	*+ve*	*−ve*	*+ve*	*−ve*	*+ve*	*−ve*
2N	203	770	665	281	406	548	91	43	73	49	59	94
>2N	4	87	90	42	23	69	0	2	0	0	1	0
	$P<0.005$		NS*		$P<0.005$		NS**		NS		NS**	

NS, nonsignificant.
*P equal to 0.61; χ^2 test.
**P equal to 0.10 and 0.20 respectively; Fisher's exact test.

Table 4 Phenotypic characteristics of cells and chromosome 18 status

Chromosome 18	*Columnar*						*Goblet*					
	MUC2		*MUC5AC*		*MUC6*		*MUC2*		*MUC5AC*		*MUC6*	
	+ve	*−ve*	*+ve*	*−ve*	*+ve*	*−ve*	*+ve*	*−ve*	*+ve*	*−ve*	*+ve*	*−ve*
2N	72	269	167	170	135	199	25	14	16	11	6	26
>2N	4	16	11	25	7	24	0	0	0	0	2	0
	NS*		$P<0.05$		NS*		NS		NS		NS**	

NS, nonsignificant.
*P equal to 0.9 and 0.051, respectively; χ^2 test.
**P equal to 0.061; Fisher's exact test.

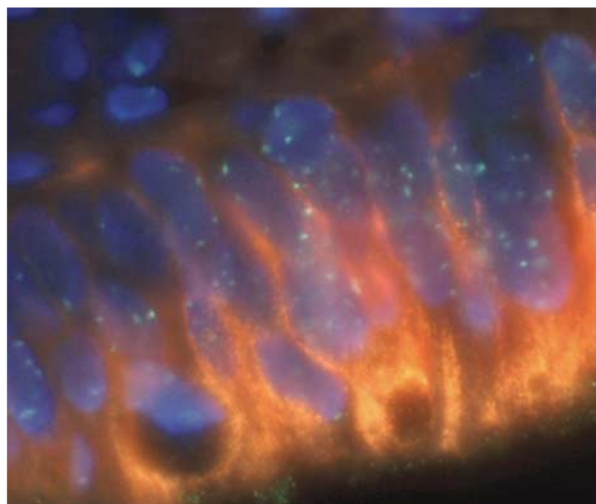

Figure 1 IF-FISH assay for chromosome 7 and MUC5AC. MUC5AC producing columnar cells (red/orange) with gain of chromosome 7 (green).

$P<0.05$). For the phenotypic characterization of the cells with gain of chromosome 18 in the two cases detected, we analyzed 400 (200 per case) cells per IF-FISH assay (Table 4). In IF-FISH assay for MUC2, 361 columnar and 39 goblet cells were evaluated. MUC2 was detected in 76/361 (21%) columnar and in 25/39 (64%) goblet elements. No difference was observed between the frequency of chromosome 18 gains in columnar cells with and without MUC2 (4/76 (5%) *vs* 16/285 (6%)). In IF-FISH assay for MUC2, gain of chromosome 18 was not detected in goblet elements (0/39). The IF-FISH assay for MUC5AC evaluated 373 columnar and 27 goblet elements. MUC5AC was observed in 178/373 (48%) columnar and in 16/27 (59%) goblet elements. In columnar cells, chromosome 18 gain was more frequent in elements without MUC5AC (25/195 (13%) *vs* 11/178 (6%) $P<0.05$). Gain of chromosome 18 was not detected in goblet cells (0/27) by IF-FISH for MUC5AC. The IF-FISH assay for MUC6 evaluated 365 columnar and 35 goblet cells. MUC6 was expressed in 142/365 (39%) columnar and in 9/35 (26%) goblet elements. There were no differences between the frequency of chromosome 18 gain in columnar as well as in goblet cells with (Figure 2) and without MUC6 (7/142 (5%) *vs* 24/223 (11%)) and (2/9 (22%) *vs* 0/26).

Discussion

Chromosomal analysis of Barrett's esophagus cells confirmed that clonal, either numerical or structur-

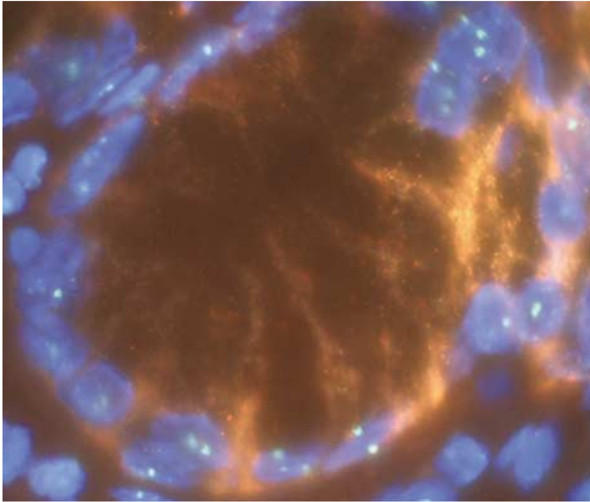

Figure 2 IF-FISH assay for chromosome 18 and MUC6. MUC6 producing goblet cells (red/orange) with gain of chromosome 18 (green).

al, abnormalities are frequent findings in nonneoplastic mucosa and demonstrated that the numerical alterations are present in both metaplastic cell lineage being independent of the cellular phenotype.

Barrett's esophagus is recognized as a phenotypically and genotypically heterogeneous structure formed by several distinct genetic clones.[23–26] This study identified different clones with distinct karyotypes in the same patient confirming this statement and illustrating the chromosomal instability present in Barrett's esophagus even in the absence of dysplasia or cancer. This instability may facilitate the expansion of clones carrying particular abnormalities with selective advantages as has been described for loss of heterozigosity at 17p and 9p.[23–26]

Conventional cytogenetics has, over other methods of chromosomal analysis, the ability to detect and characterize specific abnormalities. Its widespread routine application on Barrett's esophagus is not feasible mostly due to the difficulties in culturing nonneoplastic cells. Furthermore, the cellular heterogeneity of Barrett's esophagus limits the use of this technique because it does not allow the identification of the metaplastic population carrying the chromosomal changes. Therefore, immunophenotyping the cells resulting from successful Barrett's esophagus culture is an essential step to assure that we are selecting epithelial cells that maintain their native characteristics and not fibroblasts or epithelial-derived cells. All these difficulties may justify the small number of studies using this technique in Barrett's esophagus.[17]

In our study, the 66.6% success rate of short-term cell culture illustrates the difficulties in culturing nonneoplastic cells. The use of antibodies to cytokeratins CAM 5.2 and AE1/AE3 confirmed that the cultured cells were epithelial in origin and the positive staining for MUC5AC, MUC6 and MUC2 confirmed the presence of a mixed gastric and intestinal phenotype. This methodology, used in the 40 cases with efficient cellular growth, confirmed that the cultured cells analyzed in our study were representative of the metaplastic lineage usually present in Barrett's esophagus.

The abnormalities detected by conventional cytogenetic analysis are in accordance with the previous study of Garewal *et al*.[17] As in their paper, structural and numerical abnormalities were found. We detected structural abnormalities in 35% of the cases. Their analysis enabled us to identify some recurrent breakpoints not described previously. These recurrent breakpoints may pinpoint to important genes involved in Barrett's tumorigenesis. 1q21 was one of these breakpoints. This *locus* has not been reported previously as rearranged in Barrett's esophagus or Barrett's adenocarcinoma. However, its involvement, curiously associated with activation of the MUC1 gene, has been demonstrated in lymphomas and malignant melanoma.[27–29] A nonrandom involvement of chromosome 15 was also demonstrated in this study with breakpoints at 15q15 and 15q22. As far as we know, there is no description of genes linked to Barrett's adenocarcinoma in these particular regions. The chromosome breakpoint 15q15 has been reported as involved in breast cancers.[30] In 15q22 there are several genes that might play a role in proliferation, such as c-myc promoter-binding protein (*IRLB*), cytochrome *P*450 polypeptide 1 and –2 (*CYP1A1* and *CYP1A2*, respectively), mitogen-activated protein kinase 1 and -2 (*MAP2K1* and *MAP2K5*, respectively) and carbonic anhydrase XII (*CA12*), whose involvement has been reported in acute leukemia.[31] The nonrandom involvement of these breakpoints supports the need for further research targeting these particular chromosome locations in Barrett's tumorigenesis.

As in the study of Garewal *et al*,[17] loss of chromosome Y was a frequent numerical abnormality and we also detected recurrent gains of chromosomes 7 and 18.

Loss of chromosome Y has been reported previously as being frequent in Barrett's esophagus with a prevalence ranging from 31 to 93%.[17,32,33] However, its relationship to malignancy remains uncertain. Previous *in situ* hybridization studies in Barrett's esophagus using centromeric probes demonstrated a correlation between chromosome Y loss and increasing severity of dysplasia.[15,34,35] But, it remains to clarify its role as a cause or an effect. So far, no specific tumor suppressor genes have been mapped to the Y chromosome and Krishnadath *et al*,[36] who found no correlation between proliferation rates as detected by Ki-67 and loss of Y chromosome, did not confirm the hypothesis that its loss might confer proliferative advantage. Despite its unclear role as a side effect or a true initiating mechanism, all previous studies described chromo-

some Y loss in Barrett's esophagus adjacent to neoplasia. As far as we know this is the first study demonstrating Y loss in 14/40 cases of Barrett's esophagus without associated dysplasia or carcinoma.

Gain of chromosome 7 has been reported previously in Barrett's esophagus by Garewal *et al*.[17] The report from Walch *et al*,[33] who performed comparative genomic hybridization in Barrett's cancer and adjacent premalignant mucosa supports that this might be an important genetic event during progression to malignancy. This overrepresentation of chromosome 7 might be responsible for the increased expression of EGFR, located at 7p11.15, described in Barrett's cancer and adjacent epithelium.[37,38]

Numerical abnormalities of chromosome 18 have been described in several neoplasia, namely of the gastrointestinal tract.[39–41] In the work of Liu *et al*,[39] the gain or amplification of 18p in mucinous colorectal neoplasia was associated with aneuploidy and aggressive behavior. Stocks *et al*[41] were able to separate junctional from distal gastric tumors based on a panel of genetic abnormalities, which includes numerical aberration of 18q. Nevertheless, as far as we know, the presence of chromosome 18 numerical changes on premalignant conditions such as Barrett's esophagus was not reported.

The meaning of gross chromosomal abnormalities in a nonneoplastic epithelium is presently unknown except for particular cytogenetic alterations associated with cancer risk. Their presence may be a ubiquitous event, such as the loss of Y chromosome, or may be indicative of a genetically abnormal epithelium. The cells presenting these changes may, or may not, progress to neoplasia, but they shall be one further step in the metaplasia→dysplasia→adenocarcinoma sequence as compared with cells harboring a normal karyotype.

Nevertheless, our main goal was, not to establish the presence of cytogenetic abnormalities in Barrett's esophagus, demonstrated previously,[42–44] but to detect and to characterize the phenotype of the metaplastic cellular lineage carrying the abnormalities. For this purpose, we used numerical changes detected by cytogenetic analysis in an IF-FISH protocol. Because it is recognized that Y chromosome loss is age related and may be observed in normal tissues remaining an unspecific finding with unclear significance, we select the gains of chromosomes 7 and 18 to perform the IF-FISH analysis. Furthermore, the detection of chromosome loss by IF-FISH assay has technical pitfalls related to the section of three-dimensional structures that favor the use of chromosome gains in this assay. On the other hand, the selection of IF-FISH as a complementary technique for our cytogenetic analysis was based on its ability to recognize the distinct population involved.

In our IF-FISH study, gains of chromosomes 7 and 18 were more frequent in columnar than in goblet cells. Although goblet cells are considered a prerequisite to identify the premalignant condition,[9,18] in our IF-FISH study the chromosomal gains were more frequent in the columnar population. This suggests that columnar nongoblet as well goblet cells of Barrett's esophagus, may harbor chromosomal abnormalities and may be prone to malignant transformation. The alterations being more frequent in the predominant cell lineage of Barrett's esophagus, the columnar nongoblet elements, this population also emerges a putative candidate to adenocarcinoma precursor.

On the other hand, we found that columnar and goblet cells with chromosomal gains may display gastric as well as intestinal phenotype. This confirms that Barrett's metaplastic elements may harbor abnormalities despite its cellular phenotype. In Barrett's esophagus, as in other metaplastic epithelia, the biological meaning of cells presenting chromosomal abnormalities and aberrant phenotype is still an unclear issue. As in other tumorigenic models related to chronic inflammation such as chronic atrophic gastritis[45] and inflammatory bowel disease,[46–48] this may be related to the progressive development of simultaneous genotypically and phenotypically abnormal populations in the metaplasia→dysplasia→carcinoma sequence.

In conclusion, our study demonstrated that in Barrett's esophagus negative for dysplasia or cancer chromosomal instability is a frequent event, involves both metaplastic populations, columnar nongoblet and goblet cells, and is independent of the cellular phenotype. These challenge the consensus of considering goblet cells as the sole cell type involved in neoplastic progression in Barrett's esophagus.

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