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Keratin 7 expression as an early marker of reflux-related columnar mucosa without intestinal metaplasia in the esophagus

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Daniela Cabibi[1AEF], Eugenio Fiorentino[2AEG], Gianni Pantuso[2B], Achille Mastrosimone[2B], Cosimo Callari[2B], Matilde Cacciatore[1C], Maria Campione[1B], Francesco Aragona[1C]

[1] Department of Histopathology, University of Palermo, Italy
[2] Department of Oncology, Surgical Unit, University of Palermo, Italy

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Summary

Background: The role of Barrett esophagus in carcinogenesis is widely accepted, but the significance of esophageal columnar mucosa without histological intestinal metaplasia, known as columnar-lined esophagus, is debated.

Material/Methods: We studied 128 patients free of *Helicobacter pylori* with reflux-related symptoms and columnar mucosa in the esophagus at endoscopy, 106 patients with Barrett esophagus (referred to as the Barrett group) and 22 patients without intestinal metaplasia (columnar group). Samples from 20 subjects free of *H. pylori* were used as controls. Immunostaining for keratin 7 (KRT7), keratin 20 (KRT20), caudal type homeobox 2 (CDX2), mucin 2, oligomeric mucus/gel-forming (MUC2), and tumor protein p53 (TP53) was assessed.

Results: Samples taken 1 cm above the gastroesophageal junction showed KRT7 staining in all cases in the Barrett and columnar groups and none in the control group. Immunostaining for TP53 was absent in the control group, and more frequent in the columnar group (7, 31.8%) compared with the Barrett group (14, 13.2%, P=0.033). In the columnar group, low grade dysplasia and TP53 expression was seen in 7 of 22 biopsy specimens (31.8%) at baseline and in 4 additional specimens after 2 years, for a total of 11 specimens (50.0%).

Conclusions: The expression of KRT7 might help to explain the pathological, reflux-related nature of columnar-lined esophagus, as aberrant expression in a very early stage of the multistep Barrett esophagus progression. Expression of KRT7 may occur in basal glandular cells as a result of their multipotentiality and susceptibility to immunophenotype changes induced by reflux.

key words: **Barrett esophagus • columnar mucosa • keratin 7 (KRT7) • caudal type homeobox 2 (CDX2) • gastroesophageal reflux**

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Author's address: Cabibi Daniela, Dipartimento di Patologia Umana, Policlinico Universitario, Via del Vespro 129, 90127 Palermo, Italia, e-mail: huntermat@libero.it

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Background

Traditionally, Barrett esophagus was defined by the presence of at least 2 to 3 cm of columnar epithelium in the lower esophagus, whereas in recent literature, Barrett mucosa is defined as endoscopic findings of columnar mucosa of any length above the gastroesophageal junction (Z line) and histological findings of intestinal metaplasia with "goblet cells" [1].

In clinical practice at times, endoscopic and histological findings may not agree, for example, when a biopsy specimen from the distal esophagus shows either cardiac or fundic mucosa without intestinal metaplasia and cannot therefore be defined as Barrett mucosa [2].

Hereafter, we refer to this as columnar-lined esophagus.

Until recently, not enough attention has been dedicated to columnar-lined esophagus. The absence of goblet cells in endoscopically evident Barrett mucosa has been considered a function of sampling error [3] that could be reduced by increasing the number of biopsies at the initial diagnostic endoscopy [4], by repeating the sampling, and by assessing more sections histologically.

Nevertheless, the combination of endoscopic findings of columnar mucosa and the histologic absence of intestinal metaplasia poses clinical challenges, since the condition cannot be defined as Barrett esophagus and, without clear guidelines for clinical management, follow-up will not commonly be obtained on these patients.

Some authors [5–7] have reported the utility of the pattern of keratin 7 (KRT7) and keratin 20 (KRT20) to distinguish Barrett metaplasia from cardiac intestinal metaplasia, that is, fundic and/or cardiac mucosa with intestinal metaplasia without endoscopic evidence of Barrett esophagus. No consensus exists, however, regarding the reproducibility of the deep to superficial pattern of KRT7 and KRT20 in Barrett esophagus and its utility in the differential diagnosis of Barrett metaplasia and cardiac intestinal metaplasia [8–10]. Moreover, the immunostaining expression of KRT7 and KRT20 in columnar-lined esophagus has still to be fully described.

Caudal type homeobox 2 (CDX2), an intestinal-specific homeobox gene known to play a critical role in the differentiation and maintenance of intestinal epithelial functions, has been observed in 30% to 38% of patients with cardiac epithelium without goblet cells and in all patients with intestinal metaplasia [11,12]. Therefore, it has been suggested that CDX2 might be useful for identifying patients with Barrett metaplasia when no goblet cells are visible [12].

The aim of this study was to better understand the histogenetic and prognostic meaning of columnar-lined esophagus. We assessed KRT7, KRT20, and CDX2 immunostaining in biopsy specimens of columnar-lined esophagus from patients free from *Helicobacter pylori*, and compared the findings to patients with Barrett esophagus and to controls. We also obtained follow-up, by using mapping combined with tumor protein p53 (TP53) assessment, as previous reports have described TP53 values in low grade Barrett dysplasia and correlation with disease progression [13–15].

Material and Methods

We studied a total of 148 individuals, 128 patients and 20 controls. Written consent for studies participation was obtained from all patients. The study was approved by the ethic committee. We recruited 128 consecutive patients, who were free from *H. pylori* and with no history of previous *H. pylori* infection, who were found at endoscopy to have columnar mucosa extending from 1 cm to more than 3 cm above the gastroesophageal junction. These 128 patients formed 2 groups, 106 patients (82.8%) with Barrett esophagus (referred to as the Barrett group) and 22 patients (17.2%) with columnar-lined esophagus (referred to as the columnar group). For each patient in the columnar group, to exclude the possibility of a sampling error, endoscopy with multiple biopsies was repeated after 2 months, without any change in the endoscopic and histological findings (data not shown). At the endoscopy the gastroesophageal junction was identified as the point at which the tubular esophagus changes to a sack-like structure and the presence of hiatal hernia was assessed. The patients had been referred to the Esophageal Surgical Unit between January 2001 and May 2005 for evaluation of their suspected gastroesophageal reflux disease. All patients had 1 or more symptoms such as heartburn, regurgitation, dysphagia, otolaryngological symptoms, and asthma. All patients underwent 4-quadrant biopsies every 1 cm to 2 cm, depending on the length of the columnar-lined esophagus, and 1 biopsy below the gastroesophageal junction. Two more biopsies, one taken 1 cm below the gastroesophageal junction and one in the antrum, were performed for colorimetric test and histological assessment of *H. pylori*.

The control group consisted of 20 individuals who were free from *H. pylori*, without gastroesophageal reflux disease symptoms and lacking endoscopic findings of both reflux esophagitis and columnar mucosa in the esophagus. The squamocolumnar junction corresponded to the location of the gastroesophageal junction in all patients in the control group. For the control group, only 1 sample was obtained 1 cm below the gastroesophageal junction because only squamous epithelium was present above this point.

All histological analyses were performed by 1 of the authors (C.D.), who was masked to group assignment when performing the histological analysis. Biopsies were fixed in 10% buffered formalin and embedded in paraffin; 5 µm sections were obtained at 5 different levels and stained with hematoxylin and eosin and Alcian periodic acid Schiff (PAS) stain, to facilitate the identification of goblet cells. Intestinal metaplasia was defined as the presence of intestinal-type goblet cells stained blue with Alcian PAS stain.

Immunohistochemical studies were performed using the avidin-biotin-complex technique. The primary monoclonal antibodies used in this study were keratin 7 (KRT7, clone OV-TL12/30) and keratin 20 (KRT20, clone Ks 20.8), both obtained from Dako (Dako A/S, Glostrup, Denmark); and mucin 2, oligomeric mucus/gel-forming (MUC2), tumor protein p53 (TP53, clone DO7), and caudal type homeobox 2 (CDX2, clone AMT28), obtained from Novocastra Laboratories (Newcastle upon Tyne, United Kingdom).

Sections were cut from 10% formalin-fixed, paraffin-embedded materials, deparaffinized in xylene, and rehydrated with

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Table 1. Immunohistochemical findings in specimens from above the gastroesophageal junction in the Barrett and columnar groups and below the gastroesophageal junction in the control groups.

	Barrett group (n=106)		Columnar group (n=22)		Control group (n=20)	
	Glands	Surface	Glands	Surface	Glands	Surface
KRT7*	106 (100.0%)	106 (100.0%)	22 (100%)	18 (81.8%)	0	0
KRT20*	15 (14.2%)	90 (84.9%)	2 (9.1%)	19 (86.4%)	0	8 (40.0%)
TP53 and low grade dysplasia	14 (13.2%)	14 (13.2%)	7 (31.8%)	7 (31.8%)	0	0
CDX2	106 (100.0%)	45 (42.4%)	5 (22.7%)	5 (22.7%)	0	0
MUC2	106 (100.0%)	106 (100.0%)	0	0	0	0

Barrett group, patients with Barrett esophagus; columnar group, patients with columnar-lined esophagus (lacking intestinal metaplasia); control group, individuals without Barrett or columnar-lined esophagus. Please note that the specimens from patients in the Barrett and columnar group were obtained 1 cm above the gastroesophageal junction, and specimens from individuals in the control group were obtained 1 cm below the gastroesophageal junction.
Data are presented as number (percentage). * Focal positivity in more than one cardiac or fundic gland.
CDX2 – caudal type homeobox 2; KRT7 – keratin 7; KRT20 – keratin 20; MUC2 – mucin 2, oligomeric mucus/gel-forming; TP53 – tumor protein p53.

alcohols. Immunohistochemical assays were performed according to the manufacturer's instructions (Universal LSAAB kit, Dako). To improve the immunostaining results, samples were digested with 0.1% trypsin before incubation with KRT7, and microwaved in 10 mM citrate buffer (pH 6.0) before incubation with KRT20, TP53, CDX2, and MUC2. As a positive control for KRT7, immunostaining was performed on sections of cholangiocarcinoma that had previously proved to be positive. As positive controls for MUC2, KRT20, CDX2, and TP53, immunostaining was carried out on sections of colon adenocarcinoma that had previously proved to be positive. Negative controls without primary antibodies were included in each immunohistochemistry run.

We considered immunostaining positive for KRT7, KRT20, and MUC2 if there was at least focal cytoplasmic staining in more than 1 cardiac or fundic gland.

A nuclear staining pattern only was considered positive for CDX2 and TP53. All cases were reviewed by the 2 pathologists (D.C., F.A.), and only cases with interobserver agreement about low grade dysplasia were considered dysplastic. In keeping with previous reports on TP53 values and interobserver agreement in the diagnosis of low grade Barrett dysplasia and on correlation with disease progression, the presence of at least focal TP53 expression was used to confirm low grade dysplasia.

For patients in the columnar group, follow-up was performed after 2 years by repeating endoscopy and multiple biopsies with histological and histochemical analysis, as previously described for the baseline observations.

Statistical analysis

Statistical analysis was performed using the non-parametric Mann-Whitney test for independent samples. Differences were considered statistically significant for *P* values <.05.

Results

The 128 patients had mean age of 44 years (range 21–72). No differences were found regarding age, sex (males/females ratio: 1,5/1), smoking and alcohol use (about 60% and 10% of patients in the trhee groups, respectively). Moreover, no differences in type and duration of reflux symptoms were evidenced between the Barrett group and the Columnar group and no patients have been treated before for gastroesophageal reflux disease.

Control group

In all the subjects, free from *H. pylori*, no hiatal hernia was detected at endoscopy. All biopsies were obtained 1 cm below the gastroesophageal junction and showed normal PAS-positive fundic mucosa with no inflammation and no expression of KRT7, TP53, MUC2, and CDX2 (Table 1). Expression of KRT20 was low and focal only in the surface epithelium in 8 of 20 specimens (40.0%). Expression of KRT20 was not present in deep cardiac or fundic glands (Table 1, Figures 1A–C).

Barrett group

All the patients, free from *H. pylori*, showed the presence of hiatal hernia at endoscopy. In specimens obtained 1 cm above the gastroesophageal junction, expression of KRT7 was present in glands and surface epithelium in all cases (100%; Table 1). Expression of KRT20 was present in surface epithelium in 90 of 106 cases (84.9%) and in deep glands in only 15 of 106 cases (14.2%). Fourteen cases (13.2%) showed low grade dysplasia and expression of TP53 in columnar surface and gland epithelium. All the cases showed focal nuclear CDX2 expression in the glands, 45 (42,4%) of which showed CDX2 expression both in the glands and in the columnar surface epithelium. Expression of MUC2 was present in the cytoplasm of the goblet cells.

The biopsies performed 1 cm below the gastroesophageal junction showed no intestinal metaplasia. The expression of KRT7 was focal in the glands in 26 cases (24.5%, Table 2), 20 of which (18.8%)also showed KRT7 expression in surface epithelium. The expression of KRT20 was present on surface epithelium in 70 cases (66.0%) and focal in deep glands in only 17 cases (16.0%). No biopsy spec-

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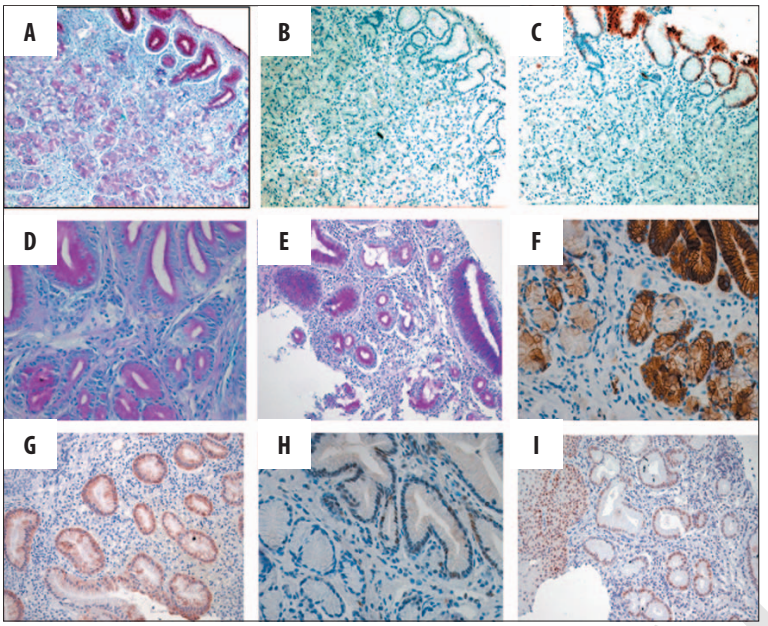

Figure 1. (**A–C**) Specimen from control subject. Fundic mucosa, 1 cm below gastroesophageal junction, was devoid of intestinal metaplasia, inflammation (Figure 1A, Alcian PAS stain, ×100), and KRT7 expression (Figure 1B, KRT7 immunostaining, ×100). Focal superficial staining for KRT20 was present (Figure 1C, KRT20 immunostaining, ×100). (**D–I**) Specimens from two subjects with columnar-lined esophagus (columnar group). Cardiac mucosa specimen was obtained 1 cm above gastroesophageal junction (Figure 1D–F–H refer to a case showing only PAS – staining glandular cells; Figure 1E–G–I refer to a case showing a few Alcian blue-staining columnar cells). Figures 1D: Cardiac mucosa, showing PAS-staining glandular cells. Figure 1E: Cardiac mucosa showing a few Alcian blue-staining columnar cells, Inflammation and clear goblet cells of intestinal metaplasia are absent in both (Figure 1D, Alcian PAS stain, ×200, Figure 1E; Alcian PAS stain, ×100). Figures 1F–I: KRT7 and TP53 immunostaining are evident in some glands that often are branching. Basal layers of squamous epithelium also show TP53 immunostaining. (Figure 1I on the left). Figure 1F, KRT7 immunostaining, ×200; Figure 1G, KRT7 immunostaining, ×100; Figure 1H, TP53 immunostaining, ×200; Figure 1I, TP53 immunostaining, ×100. KRT7 – keratin 7; KRT20 – keratin 20; PAS – periodic acid Schiff; TP53 – tumor protein p53.

imens 1 cm below the gastroesophageal junction showed MUC2, CDX2, or TP53.

Columnar group

All the patients, free from *H. pylori*, showed the presence of hiatal hernia at endoscopy. Of the 22 patients in the columnar group, the columnar mucosa extended for between 2 cm and 3 cm in 10 patients (45.5%) and for less than 2 cm in 12 patients (54.5%). At histology, weak Alcian staining was present in a few columnar cells in 7 cases (30%) but intestinal-type goblet cells were absent in all specimens (Figures 1D,E). Specimens consisted of fundic mucosa in 5 of 22 patients (22.7%), fundic-cardiac mucosa in 10 (45.5%), and cardiac mucosa in 7 (31.8%). Inflammation was absent in 9 of 22 specimens (40.1%); however, the mucosa was atrophic in appearance.

By immunohistochemistry of specimens obtained 1 cm above the gastroesophageal junction, expression of KRT7 was focal in glands epithelium in all cases, 18 of which (81.8%) also showed focal surface KRT7 expression. (Table 1; Figures 1F,G), and expression of KRT20 in surface epithelium was present in 19 specimens (86.4%). Regarding KRT20 glandular immunostaining, there were no significant differences between the 3 groups in samples from either above or below the gastroesophageal junction ($P>.05$). However, surface epithelial KRT20 immunostaining was significantly higher in both the Barrett group (66.0%, $P=.001$) and the columnar group (86.4%, $P=.002$), compared with the control group (40.0%).

TP53 was focally present in nuclei of columnar surface and gland epithelium in 7 specimens (31.8%) (Figures 1H,I). For TP53 immunostaining in glands, higher levels of TP53 staining were seen in the columnar (31.8%) versus control groups (0%, $P=.006$) and the columnar (31.8%) versus Barrett groups (13.2%, $P=.033$); however, there was no significant difference in TP53 staining between the Barrett and control groups (13.2% versus 0%, $P>.05$).

Most of the glands with KRT7 and TP53 staining showed low grade dysplasia, type II or hyperplastic dysplasia (16), consisting of mild branching (Figure 1F), pale and inconspicuous cytoplasm of the columnar cells, decreased cytoplasmic mucus production, and enlarged, rounded, vesicular nuclei with evident nucleoli. Inflammation was often absent (Figures 2A,B). Figure 2C shows TP53 immunostaining in nuclei of glandular cells. In contrast, Figure 2D shows a morphologically normal gland present in the same specimen.

TP53 expression was focally present in the squamous basal layers of all the columnar and Barrett cases in which squamous epithelium was evident.

In some cases, KRT7 expression was very focal and was limited to cystically dilated glands, often showing weak Alcian staining in a few columnar cells. In these cases, KRT7 expression was limited or more evident in the basal layer of the glandular epithelia (Figures 2E,F).

Five of 22 specimens (22.7%) in the columnar group showed CDX2 expression both in the columnar surface and gland epithelium from specimens taken above the gastroesophageal junction. (3 of 5 being P53 positive at the same time).

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Table 2. Immunohistochemical findings in specimens from below the gastroesophageal junction in the Barrett, columnar, and control groups.

	Barrett group (n=106)		Columnar group (n=22)		Control group* (n=20)	
	Gland	Surface	Gland	Surface	Gland	Surface
KRT7	26 (24.5%)	20 (18.8%)	3 (13.6%)	3 (13.6%)	0	0
KRT20	17 (16.0%)	70 (66.0%)	0	19 (86.4%)	0	8 (40.0%)
TP53 and low grade dysplasia	0	0	0	0	0	0
CDX2	0	0	0	0	0	0
MUC2	0	0	0	0	0	0

Barrett group, patients with Barrett esophagus; columnar group, patients with columnar-lined esophagus (lacking intestinal metaplasia); control group,* individuals without Barrett or columnar-lined esophagus. Information presented here for the control group is the same as in Table 1. Please note that the specimens from all subjects were obtained 1 cm below the gastroesophageal junction.
Data are presented as number (percentage).
CDX2 – caudal type homeobox 2; KRT7 – keratin 7; KRT20 – keratin 20; MUC2 – mucin 2, oligomeric mucus/gel-forming; TP53 – tumor protein p53.

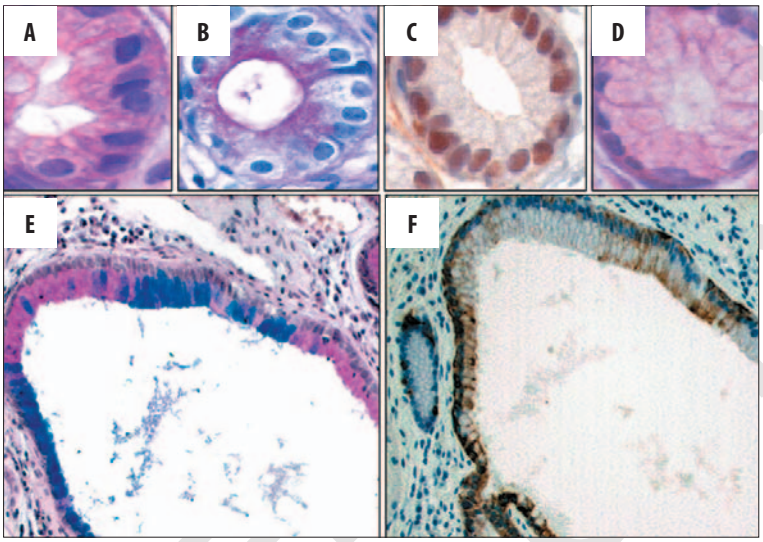

Figure 2. Specimen from patient with columnar-lined esophagus. (**A–C**) Low grade dysplasia, type II (hyperplastic dysplasia). The gland epithelium consists of columnar cells with enlarged, rounded, vesicular nuclei, evident nucleoli, inconspicuous cytoplasm (Figure 2A, hematoxylin & eosin stain, ×400), and small apical amounts of PAS-positive mucus (Figure 2B, Alcian PAS stain, ×400). Inflammation and intestinal metaplasia are absent. Nuclear staining for TP53 is present (Figure 2C, TP53 immunostaining, ×400). (**D**) For contrast, note normal gland epithelium with small basal nuclei and abundant mucus-producing cytoplasm (hematoxylin & eosin stain, ×400). (**E,F**) Cystically dilated glands, showing weak Alcian blue staining in a few columnar cells (Figure 2E, Alcian PAS stain, ×200) and focal KRT7 staining (Figure 2F, KRT7 immunostaining, ×200), more evident in the basal cell layer. KRT7 – keratin 7; PAS – periodic acid Schiff; TP53 – tumor protein p53.

With regard to CDX2 immunostaining, statistical analysis showed a significant difference between the Barrett and control groups (100% versus 0%, P=.001) and between the columnar and control groups (22.7% versus 0%, P=.025).

The specimens in the columnar group lacked clear cytoplasmic MUC2 expression. A few cases showed weak Alcian blue staining in the apex of a small number of columnar cells and very weak apical staining for MUC2, but because staining was not evident in the cytoplasm, the specimens were considered negative. With regard to MUC2 cytoplasmic immunostaining in glands, a significant difference was found between the Barrett and columnar groups (100% versus 0%), and between the Barrett and control groups (100% versus 0%), but not between the columnar and control groups.

The biopsies performed 1 cm below the gastroesophageal junction did not show intestinal metaplasia. Expression of KRT7 was focally present both in surface and gland epithelium in 3 of 22 specimens (13.6%, Table 2). There were no statistically significant differences in KRT7 staining between the Barrett and columnar group, and the columnar and control groups (P>.05). However, expression of KRT7 was higher in the Barrett group compared with the control group (P=.013). Weak KRT20 expression was present on the surface epithelium in 19 of 22 specimens (86.4%) but not present in deep glands. No biopsy specimens from below the gastroesophageal junction showed MUC2, CDX2, or TP53 immunostaining in the 3 groups.

During the 2-year period, 8 patients (36.4%) in the columnar group developed true Barrett as evidenced by the appearance of intestinal metaplasia and immunostaining for MUC2 and CDX2 (4 of which were associated with dysplasia and TP53 staining). Figures 3A and 3B show baseline observations in a specimen of fundic mucosa with weak Alcian staining and focal KRT7 expression in a few columnar cells with dilated glands, while Figures 3C–F show ob-

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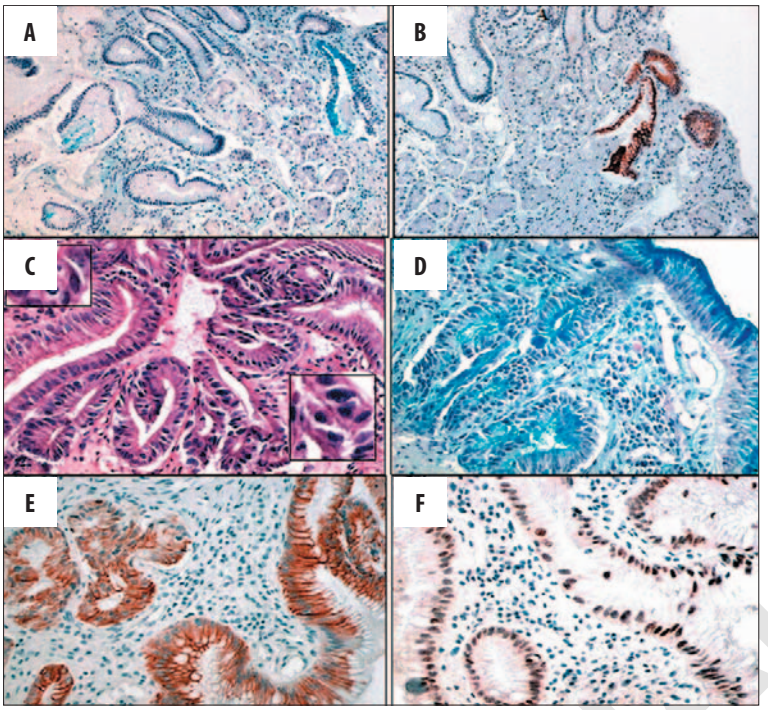

Figure 3. Specimen from patient with columnar-lined esophagus. (**A,B**) Baseline observation: weak Alcian staining (Figure 3A, Alcian PAS stain, ×100) and very focal KRT7 staining (Figure 3B, KRT7 immunostaining, ×100) in a few columnar cells present in cystically dilated glands. (**C–F**) Follow-up after 2 years in the same patient, showing dysplasia (Figure 3C, hematoxylin & eosin, ×400), intestinal metaplasia (Figure 3D, Alcian PAS stain, ×400), diffuse cytoplasmic KRT7 staining (Figure 3E, KRT7 immunostaining, ×400), and nuclear TP53 immunostaining (Figure 3F, TP53 immunostaining, ×400). The inserts in Figure 3C show nuclear changes and mitoses. KRT7 – keratin 7; PAS – periodic acid Schiff; TP53 – tumor protein p53.

Table 3. Baseline findings of 22 patients with columnar – lined esophagus.

	IM/MUC2	CDX2–	CDX2+	Total
Low grade dysplasia – TP53–	0	13	2	15 (67.8%)
Low grade dysplasia + TP53+	0	4	3	7 (31.8%)
Total	0	17 (77.3%)	5 (22.7%)	22

MUC2 – mucin 2, oligomeric mucus/gel-forming; CDX2 – caudal type homeobox 2; IM – intestinal metaplasia; TP53 – tumor protein p53.

Table 4. Two-year follow-up of 22 patients with columnar-lined esophagus.

	IM/MUC2– CDX2–	IM/MUC2+ CDX2+	IM/MUC2– CDX2+	Total
Low grade dysplasia -TP53-	5	4	2	11 (50%)
Low grade dysplasia -TP53+	4	4	3	11 (50%)
Total	9	8	5	22
	CDX2– = 9 (40.9%)	CDX2+ = 13 (59.1%)		

MUC2 – mucin 2, oligomeric mucus/gel-forming; CDX2 – caudal type homeobox 2; IM – intestinal metaplasia; TP53 – tumor protein p53.

servations at follow-up with the appearance of dysplasia, intestinal metaplasia, and diffuse KRT7 and TP53 immunostaining.

In the follow-up a total of 13 specimens (59.1%) in the columnar group showed CDX2 nuclear expression, and a total of 11 specimens (50.0%) showed TP53 expression in dysplastic cells. The 2 latter conditions often occurred independently from the appearance of intestinal metaplasia and of MUC2 staining (Tables 3,4).

Discussion

Follow-up of patients with Barrett esophagus is strongly recommended since it has been universally accepted that Barrett adenocarcinoma does not arise *de novo* but follows an established sequence from intestinal metaplasia through dysplasia to adenocarcinoma. The presence of intestinal metaplasia with goblet cells has been considered the most significant change for the diagnosis of Barrett esophagus, a fact emphasized by the dictum "no goblets, no Barrett's" [17].

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Consequently, despite endoscopic evidence of columnar mucosa, which can be extremely long and extend above the Z line in certain patients, the histological absence of goblet cells does not permit the pathologist to diagnose Barrett esophagus, and the hypothesis of a sampling error is put forward [3]. Moreover, the optimal management of such patients has not been clearly reported.

Several recent studies hypothesize the utility of CDX2 in the assessment of columnar-lined esophagus [12], that CDX2 immunostaining might be useful for identifying patients with Barrett metaplasia when no goblet cells are found in biopsies from the columnar-lined esophagus. Nevertheless, the low percentage of CDX2-positive cases reported by previous studies, ranging from 30% [11] to 38% [12], leaves the problem of the diagnosis and management of a majority of patients still unsolved.

Previous studies [5,6] have demonstrated the utility of KRT7 and KRT20 in distinguishing Barrett metaplasia from cardiac intestinal metaplasia, whereas others have failed to confirm this claim [8–10]. Some authors state that the 2 conditions are virtually indistinguishable since they are biologically related [9]. To the best of our knowledge, the expression of KRT7 and KRT20 in columnar-lined esophagus has still not been fully described. In keeping with previous studies [5,18,19], which report that normal cardiac and fundic glands do not stain for KRT7 and KRT20, and do not stain or only weakly stain for KRT20 in superficial foveolar epithelium, in our study, we considered as positive all cases showing at least focal staining for KRT7 or KRT20 in more than 1 cardiac or fundic gland. With regard to KRT20, both in Barrett esophagus and columnar-lined esophagus cases, more surface immunostaining was observed compared with the control group, but there was no significant difference between the 3 groups in glandular KRT20 expression. For this reason, we will not further discuss KRT20.

Expression of KRT7 was similar in all cases of Barrett esophagus and columnar-lined esophagus, with the same distribution pattern consisting of KRT7 staining of surface epithelium and both superficial and deep glands, as previously described in Barrett's cases [5,6]. In contrast, the samples obtained 1 cm below the gastroesophageal junction in patients in the control group and in the majority of patients in the columnar and Barrett groups did not show KRT7 immunostaining, in concordance with the previously-mentioned data regarding normal gastric glands [5,18,19]. These observations make it unlikely, at least for most of the columnar-lined esophagus cases, that a sampling error is involved and support the hypothesis that columnar-lined esophagus, like Barrett esophagus, arises from multipotential stem cells in the esophagus and not from migration of gastric mucosa, which is immunohistochemically different.

We observed several specimens of columnar-lined esophagus consisting of fundic mucosa in which focal KRT7 staining was localized in cystically dilated glands (Figures 2F,G, 3A,B). Follow-up showed the successive appearance of cardiac mucosa and intestinal metaplasia in some of these (Figure 3D). In agreement with what has been previously suggested for "reflux carditis" [20,21], these findings lead to the hypothesis that in cases of columnar-lined esophagus that are *H. pylori*-free, fundic mucosa expressing KRT7 might also be an acquired reflux-related finding, probably developing before "reflux carditis."

This sequence from fundic mucosa with KRT7 staining in cystically dilated glands to cardiac mucosa to intestinal-type mucosa might be similar to the sequential morphological changes described in the duodenogastric stoma of rats after surgery inducing duodenal-gastric reflux [22]. The absence of inflammation in 9 of 22 cases is in keeping with observations [23] that in reflux-related disease, intestinal metaplasia may develop as a result of the direct toxic effects of acid, nitrosamines, and bile without a primary role for inflammation. In our study, no patient had a history of *H. pylori* infection, so we can reasonably exclude a role for *H. pylori* in inducing KRT7. We could also hypothesize that KRT7, never present in normal fundic samples, is an aberrant expression, similar to that previously described for KRT20 expression in the extrahepatic bile tract [24]. It might represent a very early reflux-related stage, earlier than CDX2 expression, in the multistep progression leading to Barrett metaplasia, according to the sequence:

Columnar mucosa → KRT7 → CDX2 → intestinal metaplasia → dysplasia.

In our opinion, the validity of this sequence hypothesis is supported by the follow-up showing the subsequent appearance of intestinal metaplasia and CDX2 in 8 of 22 patients (36.4%) with columnar-lined esophagus, 4 of which also showed the appearance of low grade dysplasia and TP53 immunostaining, in spite of the absence of intestinal metaplasia in the first biopsies. The features of low grade type II dysplasia raise the question of differential diagnosis with atypical regenerative changes. As regenerative changes are usually accompanied by inflammation, the histological changes that we observed, occurring without inflammation but together with TP53 immunostaining, suggest the presence of the so-called "low grade dysplasia type II" or "hyperplastic dysplasia" [16].

The meaning of non-goblet cells in the malignant transformation of Barrett esophagus is still debated [25–28].

With regard to intestinal metaplasia, our observations are in keeping with the statements of Tastuta and colleagues, [29] about the crucial role of CDX2 in directing intestinal-type differentiation of the columnar cells of the esophageal mucosa, since we found CDX2 expression in all Barrett esophagus cases.

Moreover, our MUC2 findings are in keeping with the observations of Chaves and colleagues [30], who reported that MUC2 expression "was restricted to Barrett's cases and was more frequent in areas with intestinal metaplasia. Columnar-lined esophagus without intestinal metaplasia did not express MUC2."

Nevertheless, we found that some columnar-lined esophagus cases showed TP53 nuclear expression and low grade dysplasia, despite the absence of intestinal metaplasia and lack of MUC2 and CDX2 expression. These findings might indicate another hypothetical pathway consisting of:

Columnar mucosa → KRT7 → dysplasia (TP53).

In keeping with Chaves and colleagues [30], it is noteworthy that the appearance of dysplasia and TP53 in follow-up was not always linked to the presence of MUC2 or CDX2 expression or intestinal metaplasia.

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The KRT7 expression in the gland basal cells may indicate that these cells are more susceptible to the changes induced by a pathological reflux stimulus, due to their multipotentiality. Furthermore, KRT7 expression is evident before CDX2 and MUC2 expression, and independent of it.

As the distribution of goblet cells, considered indispensable for the diagnosis of Barrett esophagus, is patchy, we cannot say how many of the columnar-lined esophagus cases represent underdiagnosis of Barrett esophagus because of insufficient sampling. Nevertheless, it could be hypothesized that, as a marker of reflux-related damage in the columnar-lined esophagus devoid of goblet cells, KRT7 expression may alert us to continue the long-term observation of patients who would not otherwise be followed. To the best of our knowledge, this is 1 of the first studies in which KRT7 expression is used not as an aid to differentiate Barrett esophagus from cardiac intestinal metaplasia, but as an early marker of reflux-related damage, useful for reaching a clearer understanding of the true pathologic nature of *H. pylori*-free columnar-lined esophagus. These preliminary results, reporting the presence of dysplasia associated with TP53 immunostaining in 50% of cases in a 2-year period, indicate that a surveillance program should be suggested for such patients. A larger number of cases with longer follow-up must be studied to establish the risk of progression and the optimal management of these patients.

Conclusions

KRT7 is usually expressed in Columnar lining esophagus, despite the absence of intestinal metaplasia and MUC2 staining. KRT7 aberrant expression might help to understand the pathological, reflux-related nature of columnar lining oesophagus. Early CK7 expression is evident in the basal cells of the columnar epithelia, probably more susceptible to immunophenotype changes induced by a pathological reflux stimulus, due to their multi-potentiality. The presence of p53 and mild dysplasia in baseline biopsies and in two years follow-up biopsies suggests that columnar lining oesophagus may be involved in the multistep progression of Barrett's Oesophagus.

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