

Platelet 12-lipoxygenase and stem cells in Barrett's esophagus

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Abstract. Esophageal adenocarcinoma has shown a significant increase in incidence in recent years. It is thought that the development of gastroesophageal reflux disease (GERD), followed by columnar-lined esophagus and the development of dysplasia, leads to invasive adenocarcinoma. The exact pathogenesis of this process, the diagnosis and differentiation of the metaplastic and dysplastic esophageal lesions have yet to be determined. The purpose of this immunohistochemical study was to investigate the expression of pro-tumorigenic enzyme platelet 12-lipoxygenase (p12LOX) using two new available antibodies in non-dysplastic and dysplastic Barrett's esophagus. The stem cell markers nestin, CD117 and CD44, were then evaluated. The comparative group included GERD carditis, gastric intestinal metaplasia and colorectal adenoma. The overexpression of p12LOX detected by two specific antibodies in the non-dysplastic and dysplastic Barrett's mucosa clearly demonstrated that this enzyme plays an important role in the development of esophageal adenocarcinoma.

Introduction

Cases of adenocarcinoma in Barrett's esophagus (BE) are on the increase in Western countries. Clinical and histological studies suggest a successive progression from gastroesophageal reflux disease (GERD) to columnar-lined esophagus, also known as BE, to dysplasia with a high risk of malignancy. The pathogenesis of this sequence has yet to be completely elucidated, since more cases of BE, dysplasia and adenocarcinoma were recently found among patients without GERD symptoms than those that underwent endoscopy because of GERD (1).

This result depends on the identifying criteria indicative for BE used by the endoscopist who obtains the biopsy. The management of BE remains controversial. Various guidelines exist and the international consensus over issues, such as recognition of short-segment disease and surveillance policies for uncomplicated and dysplastic disease, is lacking (2).

The histological diagnosis of BE involves the presence of columnar epithelium with goblet cells in the esophageal mucosa. Dysplasia-intraepithelial neoplasia (IEN) serves as a morphological marker for increased cancer risk. The most important diagnostic markers of IEN are poor maturation, excessive crowding of glands and cytonuclear features. It is believed that the development of BE with intestinal goblet-type cells is related to the process of proliferation and differentiation of pluripotent epithelial stem cells in response to local injury, chronic inflammation as well as the repair process being altered (3-5). The practical utility of mucin stainings, endocrine cell count, assessment of cell proliferation (Ki-67 and PCNA), expression of EGF, TGF α and p53 is limited regarding the diagnosis and differentiation of dysplastic and non-dysplastic BE (6,7).

One of the factors related to esophageal adenocarcinoma pathogenesis is an aberrant arachidonic acid (AA) metabolism through cyclooxygenase (COX) and 5- and 12-lipoxygenase (5- and 12-LOX). Numerous published studies are related to the expression of stem cell markers and pro-tumorigenic enzymes 5- and 12-LOX in BE. Findings of these studies showed a positive expression in esophageal adenocarcinoma, with inconsistent results in other lesions of esophago-gastric junction mucosa (4,8,9). This study aimed to assess platelet 12-lipoxygenase (p12LOX) and stem cell markers in BE mucosa and other gastro-intestinal mucosal lesions.

Materials and methods

Patients. Between 2005 and 2007, endoscopic biopsies were obtained from 110 patients, aged 33-71 years, with a clinical and endoscopic diagnosis of GERD and the suggestion of BE, in the Department of Gastroenterology, Medical University of Gdańsk, Poland. The pathological examination of gastro-esophageal junction biopsies performed by two independent pathologists confirmed the initial diagnosis in 19 cases. Of the immunohistochemical examinations performed in the BE cases, 5 of BE

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Table I. The positive immunoreactivity of p12LOX, CD44 and nestin in Barrett's metaplasia, GERD carditis and colorectal adenomas.

Antibodies	No. of patients	p12LOX ab7212	p12LOX ab7225	CD44	Nestin
Barrett's mucosa	19	19	18	11	10
Barrett's dysplasia	5	5	5	5	5
Gastric carditis	10	3	1	1	1
Gastric intestinal metaplasia	17	3	3	3	3
Colorectal adenoma	10	7	1	0	0

GERD, gastroesophageal reflux disease.

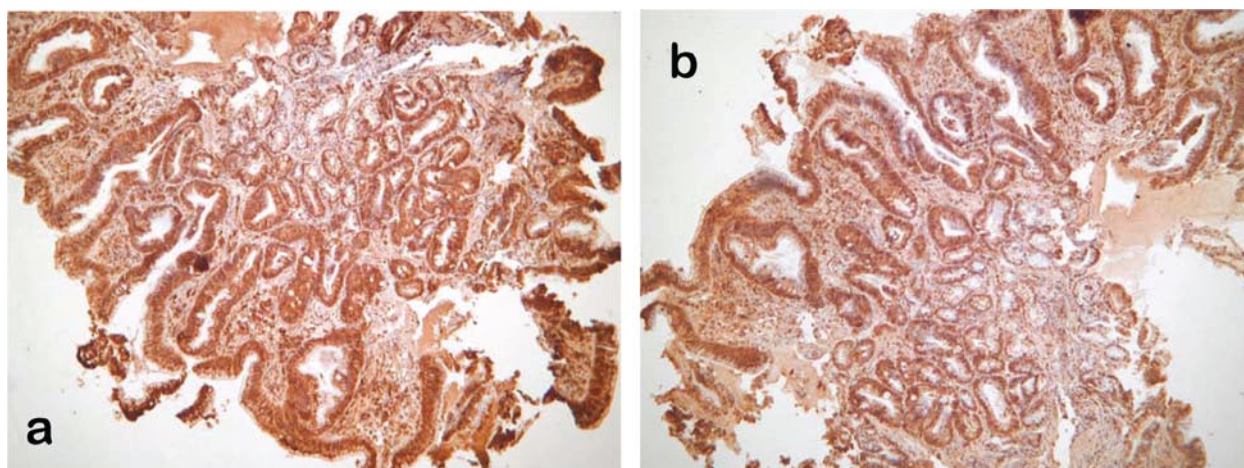


Figure 1. Strong immunostaining for 12-LOX with (a) ab7212 and (b) CD44 in Barrett's mucosa.

with low-grade dysplasia, 10 of endoscopically suggested BE without pathological confirmation (gastric carditis), 17 of gastric mucosal intestinal metaplasia and 10 of sporadic colorectal low-grade adenomas were included in the study.

Antibodies and proteins. The p12LOX antibodies were developed based on whole-length recombinant human enzyme with 7212 being rabbit, polyclonal and 7225 murine, monoclonal, anti-human antibodies (American Diagnostica, Inc.; clones 12.05 and 25.20, dilution 1:200 and 1:300, respectively). The new antibodies were checked for cross-reactivity against all human lipoxygenases. The recombinant enzymes, except for p12LOX from our laboratory (5), were generous donations from Dr T. Holman, University of California, Santa Cruz, CA, USA (5LOX, 15LOX-1 and 15LOX-2), and Dr A. Brash, Vanderbilt University Medical Center, Nashville, TN, USA (12R-LOX and eLOX3). The antibodies for nestin (196908, 1:50), CD44 (M7082, 1:50) and CD117 (K4011, 1:400) used in this study were from Dako, Dakopatts, Denmark. The tissues of gastric adenocarcinoma were used for the positive controls.

Immunohistochemistry. Paraffin-embedded tissue blocks were available for immunohistochemical evaluation in all 110 cases. Standard avidin-biotin-peroxidase complex technique was used for immunohistochemistry performed on 4- μ m paraffin

sections of formalin-fixed, paraffin-embedded tissue. Antigen retrieval in heated citrate buffer at pH 6.0 with an incubation time of 30 min was applied to the antibodies. The immunoreactivity was scored on a 3-point scale, with 1+ (low) reactivity in <10% of the epithelial cell population, 2+ (moderate) in 10-40% of cells and 3+ (high) in >40% of cells.

Results

The immunoreactivity of the tested antibodies varied. Stem cell marker CD117 was completely negative. The positive immunoreactivity of the remaining antibodies is collated in Table I. The majority of the cases of Barrett's mucosa showed moderate to high immunoreactivity with p12LOX antibodies (Fig. 1a) and more than half of the cases were immunopositive for stem-cell antibodies CD44 (Fig. 1b) and nestin. The highest immunoreactivity was observed in dysplastic BE mucosa. The 5 cases of Barrett's with IEN showed high positive immunoreactivity with p12LOX, CD44 and nestin antibodies.

In the comparative group, few clinically suspicious cases not morphologically confirmed for BE showed mild focal immunopositive reactions. A total of 3 of 17 cases in the gastric mucosal intestinal metaplasia cases showed focal immunopositivity for the tested markers restricted to the foci of low-grade mucosal dysplasia. The majority of the colorectal adenomas

with low-grade dysplasia showed mild positive immunostaining for p12LOX ab7212 (rabbit, polyclonal), whereas p12LOX ab7225 (murine, monoclonal) was positive in only 1 case with negative stem cell markers.

Discussion

The diagnostic incidence of BE in the endoscopic biopsy material obtained from the OG junction mucosa of patients with GERD, endoscopically suspected for BE, (13%) is comparable to the incidence reported by other studies (1,10). Our diagnostic criteria of BE and lesions suggestive for GERD gastric carditis are the same as those of Montgomery (10).

Lipoxygenases (LOXs) are significant enzymes that metabolize AA to hydroxyl-eicosatetraenoic acids (HETE) and leukotrienes involved in inflammatory and carcinogenic processes. Platelet 12-LOX metabolite 12S-HETE affects cell proliferation and apoptosis on the signal transduction pathway mediated by ERK (11). Limited information related to the role and expression of LOX in BE is currently available. 5-LOX showed immunohistochemical overexpression during esophageal adenocarcinogenesis (12). 5- and 12-LOX are regarded as pro-tumorigenic enzymes in colonic carcinogenesis and their overexpression was also described in various types of cancer [(13-16) and references therein]. The new antibodies developed for this study were the first on the market with specificity for the whole-length enzyme and with proven lack of any cross-reactivity with other human LOXs. Our studies have shown the usefulness of these new antibodies for immunohistochemical studies of paraffin-embedded samples in melanoma, prostate, uterine and kidney cancers (data not shown) in addition to the gastrointestinal samples discussed in this study.

Our findings showed extremely high immunoreactivity of the two p12LOX antibodies in non-dysplastic BE and Barrett's dysplasia, thereby confirming the pro-carcinogenic activity of platelet 12-LOX and suggesting the diagnostic utility of the two antibodies in GERD and BE.

CD44 is a cell surface molecule enrolled in cell-cell and cell-extracellular matrix protein interactions. In particular, its spliced variants 5 and 6 have been shown to play a role in the progression of certain tumors, including gastric carcinoma. According to Menges *et al* (9), the expression of CD44 noted in Barrett's carcinoma did not increase compared to non-dysplastic BE and was completely negative in gastric mucosa. Other investigators (8,17) showed that CD44 progressively increases in Barrett's dysplasia and adenocarcinoma.

Stem cell markers CD44 and nestin, as shown in our study, are potential markers of malignant transformation in BE, similar to intestinal metaplasia of the stomach (17,18). Our study results also showed that p12LOX and stem-cell immunoreactivity is much higher in BE when compared to other gastrointestinal mucosal cancer precursor lesions and suggests a more active pre-neoplastic transformation of Barrett's mucosa.

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