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Original article

Expression profiles of Annexin A1, formylated peptide receptors and cyclooxigenase-2 in gastroesophageal inflammations and neoplasias

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ABSTRACT

The anti−inflammatory protein Annexin−A1 (ANXA1) is associated to tumor invasion process and its actions can be mediated by formylated peptides receptors (FPRs). Therefore, we evaluated the expression and correlation of ANXA1, FPR and cyclooxygenase−2 (COX−2) enzyme in esophageal and stomach inflammations and neoplasias. The study of proteins was performed by immunohistochemistry in biopsies of esophagitis, Barrett's esophagus, squamous cell carcinoma and adenocarcinoma of the esophagus, as well as gastritis, stomach polypus and gastric adenocarcinoma. The intensity of the expressions was evaluated by densitometry. The immunohistochemical and densitometric analyzes showed specificity for the FPR1 receptor and modulation of the ANXA1, COX−2 and FPR1 expressions in the epithelial cells in the different studied conditions. Increased immunoreactivity of these proteins was observed in cases of inflammation and stomach polypus. Interestingly, moderate immunoreactivity for ANXA1 and FPR1 but increased immunolabeling for COX−2 were observed in Barrett́s esophagus and esophageal adenocarcinomas. Also, there was reduced expression of ANXA1 and FPR1 in esophageal carcinoma but COX−2 overexpression in this tumor. There was no expression of FPR2 but ANXA1 and FPR1 expressions were positively correlated in all clinical conditions studied. Positive correlation between ANXA1 and COX−2 were also observed in inflammation conditions while negative correlation between ANXA1 and COX−2 was observed in esophageal carcinoma. Our results demonstrate the unregulated expression of ANXA1 and COX−2 in precursor lesions of esophageal and stomach cancers, reinforcing their involvement in gastroesophageal carcinogenesis. In addition, the data show that the actions of ANXA1 in the inflammatory and neoplastic processes of the esophagus and stomach are specifically mediated by the FPR1 receptor.

1. Introduction

Inflammation and tumors of the esophagus and stomach are important clinical conditions with high incidence and mortality [1–3]. It is known that in many neoplasias, inflammation plays a fundamental role in tumor initiation, progression and metastasis [2,4,5]. The pro-inflammatory cyclooxygenase-2 (COX-2) enzyme has been observed to be increased in different tumors and related to the processes of angiogenesis, tumor proliferation and metastasis [5]. Studies have shown the prognostic significance of COX-2 in esophageal adenocarcinomas and carcinomas [6–9], as well as in gastric inflammations and cancers [10–15].

In addition, investigations indicate that COX-2 can be regulated by the antiinflammatory protein annexin A1 (ANXA1) [15,16]. ANXA1 presents tumor-specific expression pattern and is related to the regulation of cell growth, tumor invasion, metastasis, apoptosis and drug resistance, being considered an important target for research related to tumor development [16–19]. But the role of ANXA1 in cancer is conflicting as the protein can be downregulated in some cancers and upregulated in others [19].

The expression of ANXA1 has already been observed in different clinical conditions of the upper digestive system, especially esophageal carcinomas and stomach adenocarcinomas [17–19]. Studies indicate loss of the protein expression in the early stages of tumorigenesis in the

Abbreviations: µm, Micrometer; Ac2-26, Mimetic peptide Ac2-26 of annexin protein; ADC, Adenocarcinoma; ANOVA, Analysis of variance; ANXA1, Annexin A1Protein; BSA, Albumina bovina; COX-2, Cyclooxygenase 2; DAB, Diaminobenzine; ERK, Extracellular signal-related kinase; FPR, Formylated Peptides Receptors; HE, Hematoxylin-Eosin; Iβ1P1, Integrin beta-1 binding protein 1; n, Sample number; p, value of p (significance of the statistical test); pH, Hydrogenion potential; R, Correlation coefficient; S.E.M, Standard error of mean; vs, Versus; °C, Celsius degree

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esophagus [20–22] and overexpression in gastric carcinogenesis [18,23–26].

Besides, since the biological functions of ANXA1 occur through the interaction of the protein with receptors for formylated peptides (FPRs), the unregulated expression of ANXA1 and FPRs has also been observed in a variety of tumors. [17,19,27–29]. However, reports on the role of ANXA1 in Barretťs esophagus, precursor metaplasia of esophageal adenocarcinoma are rare [30], and studies on the expression of the protein and its receptors in the stomach polyp are still unknown.

In view of this and the conflicting data found in the literature, in this study we investigated the expression of ANXA1 correlated to the expressions of FPRs and COX-2 in different inflammatory and tumor conditions of the esophagus and stomach.

2. Materials and methods

2.1. Biopsies

The analyzes were performed on biopsies of esophagitis, Barrett's esophagus, moderately differentiated squamous cell carcinoma and moderately differentiated esophageal adenocarcinoma, as well as gastritis, stomach polypus and moderately differentiated gastric adenocarcinoma (n = 20/group). The biopsies were obtained from the files of the Service of Pathology, Faculty of Medicine of the Integrated Colleges Padre Albino of Catanduva, Brazil, after approval of the Committee of Ethics in Research (Protocol 73/11). Fragments of normal esophagus and stomach were used as controls.

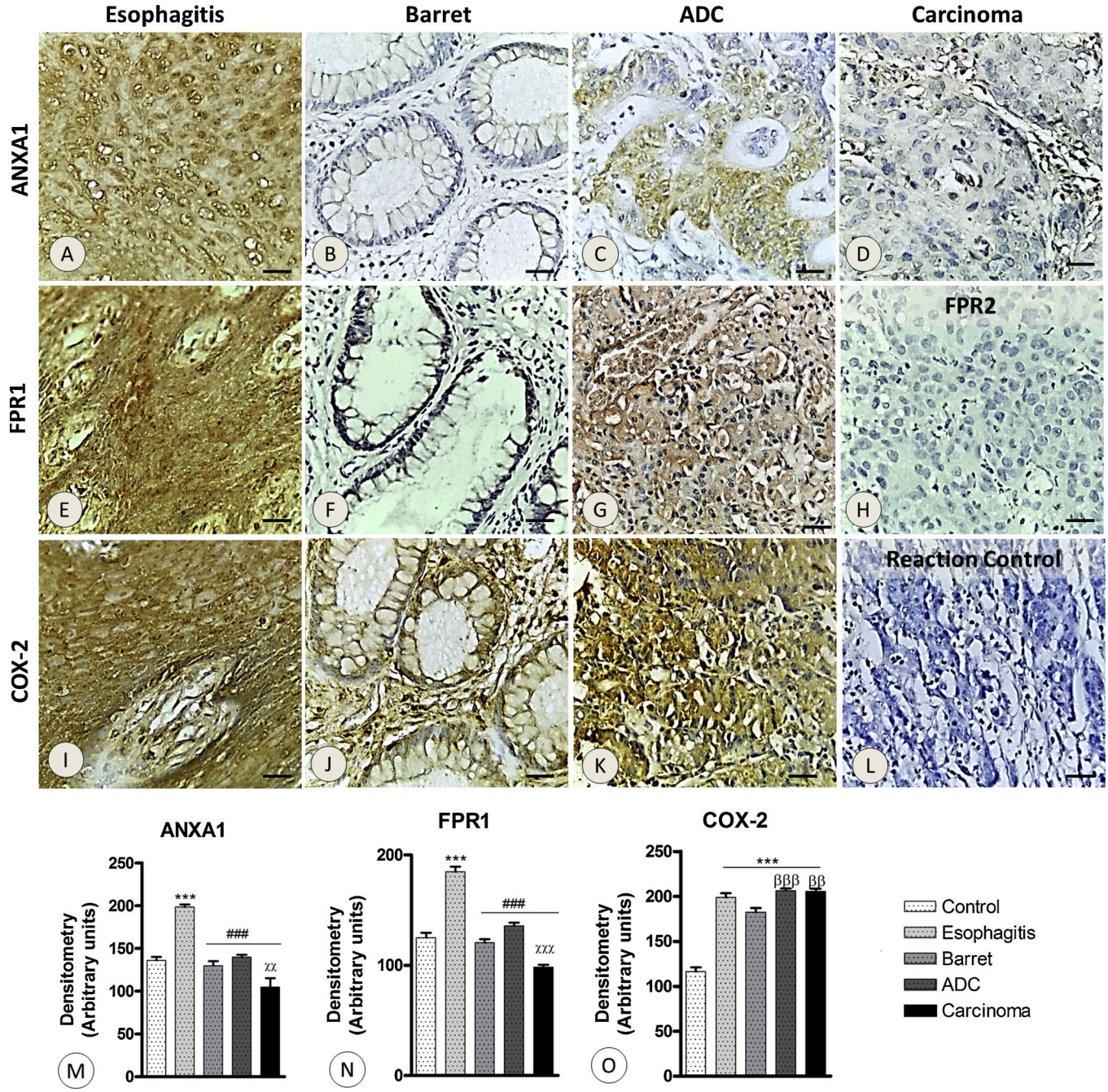

Fig. 1. Modulation of the expressions of ANXA1, FPR1 and COX-2 in esophageal inflammation, metaplasia and neoplasias. Esophagitis (A, E and I), Barretťs esophagus (B, F and J), adenocarcinoma (ADC) (C, G and K) and squamous cell carcinoma (D). Absence of immunoreactivity for FPR-2 (H) and in the control of the reaction (L). Densitometric analyzes of ANXA1 (M), FPR1 (N) and COX-2 (O). Counterstaining: Hematoxylin. Bars: 10 μm. Values expressed as mean ± S.E.M. *** $p < 0.001$ vs control; ### $p < 0.001$ vs esophagitis; ββ $p < 0.01$ and βββ $p < 0.001$ vs Barretťs esophagus; χχ $p < 0.01$vs control and ADC; χχχ $p < 0.001$ vs control, Barrett and ADC.