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Original contribution

Aurora kinase A in Barrett's carcinogenesis☆,☆☆,★

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Summary In Barrett's mucosa, both aneuploidy and *TP53* mutations are consistently recognized as markers of an increased risk of Barrett's adenocarcinoma. Overexpression of the mitotic kinase encoding gene (*AURKA*) results in chromosome instability (assessed from the micronuclei count) and ultimately in aneuploidy. Eighty-seven esophageal biopsy samples representative of all the phenotypic lesions occurring in the multistep process of Barrett's carcinogenesis (gastric metaplasia in 25, intestinal metaplasia in 25, low-grade intraepithelial neoplasia in 16, high-grade intraepithelial neoplasia in 11, and Barrett's adenocarcinoma in 10) were obtained from long segments of Barrett's mucosa. Twenty-five additional biopsy samples of native esophageal mucosa were used for control purposes. In all tissue samples, the immunohistochemical expression of both *AURKA* and *TP53* gene products was scored; and the micronuclei index was calculated. AURKA immunostaining increased progressively and significantly along with dedifferentiation of the histologic phenotype ($P < .001$). Nine of 10 Barrett's adenocarcinomas showed AURKA immunostaining. AURKA expression correlated significantly with p53 expression and the micronuclei index (both Ps $< .001$). AURKA overexpression is significantly associated with Barrett's mucosa progressing to Barrett's adenocarcinoma and contributes to esophageal carcinogenesis via chromosome instability. The identification of AURKA as a novel molecular target of cancer progression in Barrett's mucosa provides a lead for the development of new therapeutic approaches in Barrett's mucosa patients.

1. Introduction

Barrett's mucosa (BM) is generally considered a precancerous lesion, but the information available on the molecular mechanisms involved in the esophageal mucosa intestinalization and its eventual progression to cancer remains puzzling [1-6].

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★ Specific author contributions: All authors of this research paper participated directly in the planning, execution, and analysis of the study.

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Intraepithelial neoplasia (IEN) arising in metaplastic BM glands is the most reliable phenotypic marker of incipient progression to Barrett's adenocarcinoma (BAc), and IEN ablation virtually prevents invasive cancer [7,8]. No additional markers of BAc risk are currently available. As in other precancerous conditions, so too in BM, finding molecular markers that identify cancer-prone patients could significantly influence secondary cancer prevention strategies.

From a molecular viewpoint, both aneuploidy and *TP53* mutations have been significantly associated with cancer-prone BM. Aurora kinase A (AURKA; also called *STK15*, *BTAK*, *aurora-2*, *AIK1*, or *ARK1*) is a serine/threonine kinase acting as a regulator of centrosome function/duplication, mitotic entry, and bipolar spindle assembly [9-12]. As a consequence, AURKA changes imply an incomplete cytokinesis and chromosome instability (CIN) [9-13]. In experimental models in vitro and in vivo, AURKA overexpression was found to be oncogenic and consistently associated with *TP53* mutation [9-11]. *AURKA* gene amplification and overexpression of AURKA messenger RNA (mRNA) (and its protein product) have been consistently associated with human cancer [9-12]. In particular, AURKA overexpression has been documented in both esophageal columnar metaplasia [14] and adenocarcinoma [15].

This study aimed (*a*) to assess AURKA immunohistochemical (IHC) expression over the whole spectrum of phenotypic lesions involved in Barrett's carcinogenesis; (*b*) to explore the association between AURKA expression and CIN (assessed from the micronuclei [MNi] index); and (*c*) to investigate any relationship between AURKA overexpression, MNi index, and p53 expression.

2. Materials and methods

2.1. Complementary DNA microarray analysis

The Oncomine database and gene microarray analysis tool, a repository for published complementary DNA microarray data (http://www.oncomine.org) [16,17], was explored (on September 15, 2009) for aurora kinase A (AURKA, STK15, BTAK, AIKI) mRNA expression in nonneoplastic esophageal mucosa, in BM, and in BAc. The differences in AURKA expression between the said comparisons were analyzed statistically using Oncomine algorithms, which enable multiple comparisons between different studies [16-18]. Only studies with results with a $P < .05$ were considered.

2.2. Patients and samples

All cases considered in this study were collected retrospectively from the files of the Veneto region's multicenter Barrett's Esophagus Registry (EBRA, Padova unit) [19], considering tissue samples obtained from 80 consecutive (histologically proven) long-segment BM patients. In all, 87 biopsy samples were considered: 25 were of columnar IM-negative (IM−) esophageal mucosa (gastric type = GM), 25 were of columnar IM-positive (IM+) esophageal mucosa (IM), 16 were of IM+ low-grade IEN (LG-IEN), 11 were of IM+ high-grade IEN (HG-IEN), and 10 were of BAc. All 27 IEN and 10 BAc samples were obtained from 37 different patients. Twenty-five additional samples of normal (N) squamous esophageal mucosa were obtained from dyspeptic patients, with no endoscopic or histologic lesions. All the patients considered in this study gave their written informed consent.

2.3. Histologic and IHC study

All biopsy specimens were fixed in formalin and embedded in paraffin. Histologic sections 4 to 6 μm thick were stained with hematoxylin and eosin, and with Alcian-PAS. The original diagnosis was always confirmed histologically according to internationally accepted criteria [20]. The IHC stains were obtained automatically (Ventana Benchmark XT system; Touchstone, AZ) [21] for both AURKA (Epitomics Inc, Burlingame, CA; 1:100) and p53 (Immunotech, Marseilles, France; prediluted) according to the manufacturers' instructions. Sections were lightly counterstained with hematoxylin. The human breast carcinoma cell line MCF7 (in cell blocks) was used as a positive control for AURKA IHC overexpression [22]; a p53-mutated breast cancer was considered for p53 protein IHC overexpression. Negative controls were obtained by omitting the primary antibodies. IHC expression of AURKA was scored jointly by 2 pathologists (M. R. and M. F.) blinded to any clinical information. The histologic score was consistent with other validated methods [15]. In particular, AURKA IHC staining was considered positive when target cells (ie, native squamous epithelia of normal esophagus, columnar-metaplastic cells of gastric metaplasia, goblet-intestinalized cells of intestinal metaplasia, dysplastic cells of LG/HG-IEN, and neoplastic epithelia of BAc) showed unambiguous cytoplasm staining. Nuclear staining was also recorded, but was subsequently not considered. The intensity of staining was scored as absent (=0), weak (=1), moderate (=2), or strong (=3; taking the MCF7 cells for reference). The prevalence of stained cells in each lesion was scored as percentage of positive cells, that is, 0 = less than or equal to 3%; 1 = 4% to 25%; 2 = 26% to 50%; 3 = 50% to 75%; 4 greater than or equal to 75%. The scores for prevalence and intensity were then indexed (index score [Is]) as the product of the 2 scores: Is 0 = 0; Is 1 = 1 and 2 (weak), Is 2 = 3 and 4 (moderate), Is 3 = greater than 6 (strong). Nuclear staining was considered for p53, semiquantified using a 4-tiered scale based on extent of staining: 0 = less than or equal to 3%; 1 = 4% to 33%; 2 = 34% to 66%, 3 = greater than or equal to 67%.