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Original contribution

Evaluation of human tissue kallikrein-related peptidases 6 and 10 expression in early gastroesophageal adenocarcinoma☆,☆☆,★

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Summary Kallikreins are a family of serine proteases that are linked to malignancy of different body organs with potential clinical utility as tumor markers. In this study, we investigated kallikrein-related peptidase 6 (KLK6) and KLK10 expression in early gastroesophageal junction adenocarcinoma and Barrett esophagus (BE) with and without dysplasia. Immunohistochemistry revealed significantly increased KLK6 expression in early invasive cancer compared with dysplastic (P = .009) and nondysplastic BE (P = .0002). There was a stepwise expression increase from metaplasia to dysplasia and invasive tumors. Significantly higher KLK10 was seen in dysplastic lesions compared with metaplasia but not between dysplastic lesions and invasive cancers. KLK6 staining intensity was increased at the invasive front (P = .006), suggesting its role in tumor invasiveness. Neither KLK6 nor KLK10 was significantly associated with other prognostic markers, including depth of invasion, indicating their potential as independent biomarkers. Our results should be interpreted with caution due to limited sample size. There was a significant correlation between KLK6 and KLK10 expression both

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★ All authors contributed to drafting and reviewing the manuscript. Andrea Grin, Monika Tripathi, and Sara Samaan were involved in data collection, specimen collection, and immunohistochemistry interpretation. Fabio Rotundo and Kalman Kovacs were responsible for immunohistochemical staining, Mena N. Bassily led the statistical analysis. George M. Youssef, Andrea Grin, and Kalman Kovacs were responsible for the study design, data interpretation, and manuscript revision.

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at the invasive front and within the main tumor, indicating a collaborative effect. We then compared *KLK6* and *KLK10* messenger RNA expression between metaplastic and cancerous tissues in an independent data set of esophageal carcinoma from The Cancer Genome Atlas. KLK6 and KLK10 may be useful markers and potential therapeutic targets in gastroesophageal junction tumors.

1. Introduction

The incidence of gastroesophageal junction (GEJ) adenocarcinoma is rapidly increasing, particularly in the Western world. The only known precursor of this cancer is Barrett esophagus (BE), which is characterized by a change in epithelium at the GEJ into a glandular type mucosa [1,2] and is typically asymptomatic in the general population [3,4]. Advanced tumors have a poor prognosis [5], but if tumors are detected early, patients can be treated and cured by therapeutic endoscopy. Because patients with BE are prone to developing adenocarcinoma, efforts have been geared toward improving early detection and lowering cancer-related mortality via surveillance endoscopy, but various challenges reduced their efficacy [6-8]. Therefore, there is an increasing need for quantifiable biomarkers that can aid in the diagnosis and prognosis of adenocarcinoma [9,10].

Kallikreins are serine proteases that are ubiquitously expressed in various organs of the body. These genes are clinically interesting due to their utility as prognostic biomarkers in many cancers, including prostate, breast, and ovarian cancers [11-13]. Kallikrein-related peptidase 6 (KLK6) and KLK10 are members of the human kallikrein gene family of secreted serine proteases, which have been implicated as markers of poor prognosis in human malignancy [14]. Previous studies have revealed KLK6 to be up-regulated in serum of ovarian cancer patients and in gastrointestinal cancers including gastric, colorectal, esophageal, and pancreatic cancers [15-17]. It was also found to contribute to cancer pathogenesis by degrading extracellular matrix proteins, leading to angiogenesis, tumor invasion, and metastasis [18-20]. Similarly, KLK10 is up-regulated in colorectal cancer, and its methylation is associated with breast cancer [21-23]. Moreover, literature shows that there is co-expression of KLK6 and KLK10 in pancreatic ductal adenocarcinoma, colorectal carcinoma, and ovarian cancer, pointing to a possible interaction between them that might contribute to cancer pathogenesis [24-28].

Although preliminary studies have demonstrated increased expression of KLK6 in gastric cancer [16,29-31], expression patterns of KLK6 and KLK10 have not been previously examined in GEJ tumors. The objective of this study was to gain a better understanding of the expression of these 2 kallikreins in GEJ adenocarcinomas by examining their expression at the protein level in a series of early GEJ adenocarcinomas. We also examined their expression pattern and localization at the cellular and subcellular level and the potential presence of a significant correlation in their expression patterns as an indication of a synergetic function. We also correlated their expression with patient outcome and other markers associated with poor prognosis.

2. Materials and methods

2.1. Ethical issues

Throughout this project, informed, written consent has been obtained; studies have been performed according to the Declaration of Helsinki; and all procedures have been approved by the Research Ethics Board of St Michael's Hospital (Toronto, Ontario, Canada).

2.2. Patients

The study included 30 patients, 21 male and 9 female, median age of 66.3 years, who underwent endoscopic mucosal resection for early GEJ adenocarcinoma at St Michael's Hospital, Toronto, Canada. Descriptive statistics of the patients and their tumors are summarized in Table 1. Cancers were subdivided by depth of invasion: lamina propria (M1), inner/duplicated muscularis mucosa (M2), between the inner and outer muscularis mucosa (M3), into the outer muscularis mucosa (M4), and into the superficial submucosa (SM1). Histologic features associated with poor prognosis were evaluated including tumor grade, lymphovascular invasion, and tumor budding. Tumor budding was defined as present if greater than or equal to 10 "buds" at ×20 objective, with a bud defined as a single tumor cell or a cluster of less than 5 cells.

2.3. Immunohistochemical staining

The immunohistochemical staining was performed on 4-μm-thick paraffin sections of tissues fixed in buffered formalin. KLK6- and KLK10-specific rabbit polyclonal antibodies were raised in-house against KLK6 and KLK10. These antibodies were tested and showed no cross-reactivity with other members of the kallikrein-related family of peptidases. Staining was performed as previously described [25,26].

2.4. Evaluation of immunohistochemical staining

Negative controls were included for every case. A combination of a proportion score (PS) and an intensity