The dichotomy in carcinogenesis of the distal esophagus and esophagogastric junction: intestinal-type vs cardiac-type mucosa-associated adenocarcinoma

Elizabeth G Demicco¹, Alton B Farris III^{1,2}, Yoshifumi Baba³, Brian Agbor-Etang⁴, Kristin Bergethon¹, Rajni Mandal¹, Diane Daives⁴, Junya Fukuoka⁵, Michio Shimizu⁶, Dora Dias-Santagata¹, Shuji Ogino^{3,7}, A John Iafrate¹, Henning A Gaisert⁴ and Mari Mino-Kenudson¹

¹Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA;

²Department of Pathology, Emory University School of Medicine, Atlanta, GA, USA; ³Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA; ⁴Thoracic Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; ⁵Department of Pathology, Toyama Medical University, Toyama, Japan; ⁶Department of Pathology, Saitama Medical University, International Medical Center, Saitama, Japan and ⁷Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Adenocarcinoma of the distal esophagus and esophagogastric junction continues to rise in incidence. An intestinal metaplasia (Barrett esophagus)–dysplasia–carcinoma sequence induced by gastroesophageal reflux disease is well established. However, a significant number of adenocarcinomas in the vicinity of the esophagogastric junction are seen in the background of gastric/cardiac-type mucosa without intestinal metaplasia. Thus, the aim of this study was to investigate the role of Barrett esophagus (intestinal-type mucosa) in the classification and prognosis of tumors of the distal esophagus and esophagogastric junction. Clinicopathological and molecular characteristics were examined in 157 consecutively resected adenocarcinomas of the distal esophagus and esophagogastric junction and were compared between tumors arising in association with intestinal-type and cardiac-type mucosa. Intestinal-type mucosa-associated adenocarcinomas were more likely to be associated with younger age ($P=0.0057$), reflux symptoms ($P<0.0001$), proximal location ($P=0.0009$), lower T stage ($P<0.0001$), fewer nodal metastases ($P=0.0001$), absence of lymphatic ($P<0.0001$), venous ($P=0.0060$) or perineural ($P<0.0001$) invasion. Histologically, intestinal-type mucosa-associated tumors were more likely to be low-grade glandular tumors ($P=0.0095$) of intestinal or mixed immunophenotype ($P=0.015$) and express nuclear β -catenin ($P=0.0080$), whereas tumors arising in a background of cardiac-type mucosa were more frequently associated with *EGFR* amplification ($P=0.0051$). Five-year overall survival rate was significantly higher in patients with intestinal-type mucosa-associated tumors (28 vs 9%, $P=0.0015$), although no survival benefit was seen after adjusting for potential confounders. Our findings support the theory that multiple distinct pathways of tumorigenesis exist in the vicinity of the esophagogastric junction, including one in which tumors arise from dysplastic intestinal metaplasia (intestinal pathway), and one potentially involving dysplasia of the cardiac-type mucosa (non-intestinal pathway). Additional studies are warranted to further clarify their pathogenesis and the molecular mechanisms involved.

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Correspondence: Dr M Mino-Kenudson, MD, Department of Pathology, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Warren 122, Boston, MA 02114, USA.

E-mail: mminokenudson@partners.org

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The incidence of adenocarcinoma of the distal esophagus and esophagogastric junction has increased sixfold within the past 40 years in the United States and Western Europe.^{1,2} A number of studies have sought to determine if the anatomic location of these increasingly prevalent tumors could be associated with specific characteristics predictive of clinical outcome. Although some investigators found differences in gender predilection, prognosis and potential etiology after separating carcinomas of the esophagogastric junction and distal esophagus based on their anatomical relationship to the esophagogastric junction,^{3–5} others emphasized their similarities, prognosis in particular.^{6,7} Owing to these similarities in anatomic location and prognosis, the 7th edition AJCC staging manual has changed its guidelines to subsume tumors of the esophagogastric junction into those of the esophagus.⁸

Other studies have suggested that the presence of intestinal metaplasia of the esophagus, that is, Barrett esophagus, has a greater impact on prognosis. Barrett esophagus is a preneoplastic precursor lesion of adenocarcinomas of the distal esophagus and esophagogastric junction^{9,10} and is found in association with approximately 60% of esophageal adenocarcinomas.¹¹ Like Barrett esophagus, adenocarcinomas of the esophagus and esophagogastric junction are more frequently found in men over the age of 65 years, many of whom have a history of gastroesophageal reflux disease.^{12,13} Some authors have claimed that adenocarcinomas arising in association with Barrett esophagus (intestinal-type mucosa-associated adenocarcinomas) have a better prognosis than those arising in a background of gastric/cardiac-type mucosa (cardiac-type mucosa-associated adenocarcinomas).^{14,15} This has led to the assertion that intestinal-type mucosa-associated adenocarcinoma is biologically different from cardiac-type mucosa-associated adenocarcinoma. Nevertheless, some groups maintain that cardiac type-mucosa-associated adenocarcinomas are simply large tumors that have overgrown pre-existing short-segment Barrett esophagus, rather than comprising a separate entity,¹⁶ and that prognostic differences between intestinal-type and cardiac-type mucosa-associated adenocarcinomas are due to early detection of tumors on surveillance endoscopy.¹⁷

Histopathological parameters other than tumor depth and lymph node involvement have not been well studied to determine their utility as predictors of natural history or treatment outcome in patients with adenocarcinoma of the distal esophagus or esophagogastric junction. We therefore sought to investigate whether the classification of distal esophageal and esophagogastric junction tumors by the presence of Barrett esophagus could be substantiated with an analysis of histopathological phenotypes, immunophenotypes and molecular alterations previously reported to be prognostic biomarkers for adenocarcinomas of the esophagus

and stomach, as well as the relevance of these factors in tumor prognostication.

Materials and methods

Acquisition of tissue specimens and clinical information were approved by the Institutional Review Board of the Massachusetts General Hospital, and performed in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations.

Patient Population

From a prospectively collected database, a series of 272 esophagectomies and esophagogastric resections for adenocarcinoma performed between January 2000 and May 2008 were identified. Of those, patients treated with neoadjuvant chemotherapy and/or radiation therapy ($n=83$), photodynamic therapy ($n=2$) or endoscopic mucosal resection ($n=4$) were excluded. Tumors were initially classified by anatomic site, using the Siewert classification system, one of the most commonly used anatomic classification systems for tumors of the esophagogastric junction.^{3,18,19} This system includes only tumors arising within 5 cm of the esophagogastric junction, and defines the anatomic esophagogastric junction as the site where the proximal gastric rugal folds terminate and the tubular esophagus begins. Type 1 tumors are those arising with their epicenter within 1–5 cm proximal to the esophagogastric junction, whereas type 3 arise within 2–5 cm distal to the esophagogastric junction, and are considered to represent tumors of the subcardia that have spread into the esophagogastric junction from below. Type 2 tumors, those with an epicenter between 1 cm proximal and 2 cm distal to the anatomic esophagogastric junction, are thought to represent true tumors of the esophagogastric junction. For the purpose of this study, the topographic-anatomic location of tumor epicenter was determined using a combination of findings on radiographic imaging, endoscopic examination, operative reports, gross measurements, gross photographs, where available, and microscopic examination (please refer to the following section). In cases where the landmarks were completely obliterated on both gross and microscopic examination, the gross description and photographs indicating the length of the tubular esophagus and the extent of tumor involvement of both tubular esophagus and stomach were used to determine epicenter. The tumors with an epicenter located in the gastric body/fundus ($n=2$), subcardia (Siewert type 3, $n=17$) or esophagus (>5 cm proximal to the esophagogastric junction, $n=7$) involving the esophagogastric junction were excluded. The remaining 157 cases formed our study group: these consisted of adenocarcinomas

of the distal esophagus (Siewert type 1) and those of the esophagogastric junction (Siewert type 2).

Clinical information including demographics, presenting symptoms, preoperative diagnosis of Barrett esophagus (intestinal metaplasia of the esophagus confirmed by review of biopsies), surveillance for Barrett esophagus, operative methods, tumor recurrence and mortality was obtained by retrospective chart reviews. For the purpose of this study, the operative approaches were classified as: (1) Ivor–Lewis ('two-hole') esophagectomy or esophagogastrectomy (a two-stage procedure involving abdominal dissection, followed by thoracic resection); (2) thoraco-abdominal esophagectomy or esophagogastrectomy; and (3) transhiatal gastroesophagectomy.

Histopathological Analysis and Tissue Microarray Construction

After formalin fixation, the specimens were sectioned in the following manner. Tumors 3 cm or

less in diameter were entirely submitted along with columnar-lined esophagus. In tumors greater than 3 cm in diameter, at least three sections of the tumor including at least one of the tumor to normal esophagus interface and at least one of the tumor to grossly uninvolved distal mucosa were sampled. In cases with no grossly visible mass, the entire columnar-lined esophagus was submitted. Uninvolved esophagogastric junction, if present, was also sampled for microscopic evaluation in all settings. The tissue blocks were fixed in 10% buffered formalin, embedded in paraffin and cut at 5 μ m. Hematoxylin and eosin (H&E)-stained slides of each case were evaluated for tumor size, depth of invasion (pT), nodal metastases (pN, in accordance with 7th AJCC tumor staging), presence of distant metastases (pM), presence of lymphovascular invasion, large vessel (venous) invasion, perineural invasion, histological grade (pG) and histomorphological features of tumor and the background mucosa (Barrett vs non-Barrett). Intestinal metaplasia was defined as the presence of goblet cells

Figure 1 Histology and β -catenin immunostaining of adenocarcinomas of the esophagogastric junction. Examples of adenosquamous carcinoma (a), low-grade conventional adenocarcinoma (b), poorly differentiated adenocarcinoma (c), medullary carcinoma (d), mucinous carcinoma (e) and signet ring cell carcinoma (f). (g) Low-grade conventional adenocarcinoma with preserved membranous expression of β -catenin. (h) Poorly differentiated adenocarcinoma showing nuclear expression of β -catenin in $>10\%$ of the tumor cells. In this example, cytoplasmic staining of β -catenin is also present, but membranous staining is completely lost in the vast majority of the tumor cells.

Figure 1 Continued.

within columnar epithelium. The esophageal location of intestinal metaplasia and/or carcinoma was confirmed histologically using the presence of submucosal glands and/or duplication of the muscularis mucosa as esophageal landmarks. Special attention was also paid to the combined presence of squamous overgrowth, multilayered epithelium, hybrid glands and/or submucosal glands/ducts to differentiate intestinal metaplasia of the esophagus (ie, Barrett esophagus) from that of the cardia in both resection specimens and preoperative mucosal biopsies available for review.²⁰ The histomorphology of tumors was classified as adenosquamous carcinoma, low-grade conventional adenocarcinoma (tubular, papillary and/or cribriform pattern, pG 1–2), poorly differentiated conventional adenocarcinoma (solid and/or nested patterns, pG 3), medullary carcinoma (solid growth with tumor infiltrating lymphocytes), mucinous carcinoma or signet ring cell carcinoma (Figure 1). In cases exhibiting a heterogeneous morphology, the most prevalent features of the highest grade were used in statistical analyses.

Tissue microarrays were constructed of 137 cases in which tissue blocks were available. For

each case, 2–5 areas that represented each histomorphological subtype were evaluated on six 2-mm-core tissue microarrays, which also included normal gastric body/fundic mucosa, cardiac-type mucosa and Barrett mucosa as controls, as well as five cores of non-esophagogastric junction cancers on each.

Immunohistochemical Analyses

Immunohistochemical staining was performed on the tissue microarray sections, using a panel of markers previously reported to be indicators of intestinal (CDX2, CD10, MUC2)^{21–23} or gastric (MUC5AC, MUC6)^{23–26} phenotype. Nuclear β -catenin and MUC1 expression—previously reported to be prognostic biomarkers for adenocarcinomas of the stomach and esophagus^{27–29}—was also evaluated. A Ventana autostainer (Benchmark XT) was used, following the manufacturer's instructions, with appropriate dilutions of antibodies to CDX2 (CDX2-88; Biogenex), CD10 (56C6; Ventana), MUC5AC (NCL-MUC5AC; Leica), MUC6 (NCL-MUC6; Leica), MUC2 (NCL-MUC2; Leica), MUC1

(NCL-MUC1; Leica) and β -catenin (14; Ventana). Sections were counterstained with hematoxylin.

Expression of each protein was considered to be positive when more than 10% of tumor cells in a section exhibited staining in the following cellular structures: CDX2, in the nucleus; CD10, on the cytoplasmic membrane; MUC2, MUC5AC and MUC6, in the cytoplasm; MUC 1, in the cytoplasm and/or on the membrane; and β -catenin, in the nucleus (Figure 1). Protein expression was collectively evaluated in all the tumor cells seen in multiple cores from each case.

Immunophenotype was classified as: intestinal if tumors stained positively for any of CDX2, CD10 and/or MUC2, and negatively for both MUC5AC and MUC6; gastric if positive for MUC5AC and/or MUC 6, and negative for CDX2, CD10 and MUC2; and mixed if positive for any combination of both intestinal and gastric markers, or null if all the cores from a case were negative for all five markers (Figures 2 and 3).^{25,30,31}

Molecular Analyses

The overexpression of the epidermal growth factor receptors *EGFR* and *ERBB2* has also been reported in association with unfavorable outcomes in esophageal adenocarcinomas.^{32–36} Thus, we conducted FISH for amplification of *EGFR* and *ERBB2* on tissue microarray sections and/or conventional tissue sections. *EGFR* or *ERBB2* gene copy number was considered to be amplified in the presence of a gene/chromosome ratio ≥ 2 or homogeneously staining regions with ≥ 15 copies in $\geq 10\%$ of cells.³⁷

Statistics

Data are shown as prevalence (%) or mean ($\pm$ standard deviation). We used the SAS program (Version 9.1, SAS Institute, Cary, NC, USA). All *P*-values were two-sided. When multiple hypothesis testing was performed, a *P*-value significance was adjusted by Bonferroni correction to $P=0.0050$. Continuous data were compared using the *t*-test, whereas categorical data was analyzed by the χ^2 -test or Fisher's exact test. Univariate analysis was performed to identify factors that were different in tumor subtypes. Overall survival was assessed by the Kaplan–Meier method, with prognostic factors evaluated by log-rank test. To assess independent effect of Barrett esophagus background on mortality, we constructed a multivariate, stage-matched (stratified using AJCC stage (I, II, III + IV)) Cox proportional hazard model to compute a hazard ratio (HR) according to Barrett esophagus background. A backward stepwise elimination with a threshold of $P=0.10$ was used to select variables in the final model. Variables in the final model were Barrett esophagus background, sex, age, surgical approaches, anemia, surveillance, tumor size, perineural

invasion, surgical margin status, Muc1 expression and β -catenin nuclear expression.

Results

Clinical Profile

The study population consisted of 157 patients (29 women and 128 men) with surgically resected adenocarcinomas of the esophagogastric area. There were 153 Caucasians and 4 Asians. Patient age ranged from 36 to 94 years (median 65 years). There were 51 cases of Siewert type 1 adenocarcinoma and 106 Siewert type 2 (Table 1). An Ivor–Lewis procedure was performed in 70 patients, whereas a thoraco-abdominal approach was taken in 66 and transhiatal approach in 21 cases. Barrett esophagus was diagnosed preoperatively in 83 patients and was found in the resected esophagus of an additional 25.

Histopathological Characteristics

Tumor size ranged from 0.1 to 11.5 cm (average: 3.8 cm), and was greater than 3 cm in 98 (62%) cases. Of 157 tumors, 79 (50%) presented at an advanced stage (T3 + T4), 83 (53%) were found to have nodal disease and 5 (3%) had distant metastasis. Lymphovascular, venous and perineural invasion was present in 85 (54%), 36 (23%) and 67 (43%) cases, respectively, and the resection margins (usually adventitial margin) were involved in 23 (15%). Approximately half of the tumors (77, 49%) were pure low-grade conventional adenocarcinoma, whereas most of the remaining cases (71, 45%) exhibited high-grade histology (pG3). By immunohistochemistry, over half of the cases (71, 52%) exhibited an intestinal or mixed phenotype; of these, 59 also showed CDX2 nuclear expression. As for biomarkers, MUC1 expression, nuclear β -catenin and *EGFR* amplification were noted in 83 (60%), 52 (38%) and 14 (9%) cases, respectively.

Clinicopathological Characteristics of Adenocarcinomas Stratified by the Background Mucosa

Over two-thirds of the cases in this study presented with Barrett esophagus (intestinal-type mucosa-associated adenocarcinoma) (Table 2). However, only one-third of the patients with Barrett esophagus had been enrolled in surveillance endoscopy before presenting with invasive carcinoma. Patients with Barrett esophagus were more likely to be under the age of 65 years ($P=0.0057$), and have more proximally located tumors ($P=0.0009$), with a history of reflux symptoms ($P<0.0001$), but no dysphagia ($P=0.011$). In general, intestinal-type mucosa-associated adenocarcinomas were asso-

ciated with better prognostic factors, including less advanced stage ($P<0.0001$), absence of nodal metastases ($P=0.0001$), negative resection margins ($P=0.0046$), lower-grade histology (pG 1–2, $P=0.0095$) and no lymphatic ($P<0.0001$), venous ($P=0.0060$) or perineural invasion ($P<0.0001$).

Histologically, intestinal-type mucosa-associated adenocarcinomas were more likely to show a glandular morphology ($P=0.0062$), and exhibit an intestinal or mixed immunophenotype ($P=0.015$), with more frequent CDX2 expression ($P=0.020$). In terms of biomarker expression, intestinal-type

Figure 2 Intestinal (a, c, e, g, i) and gastric (b, d, f, h, j) immunophenotypes. An example of the intestinal type shows nuclear expression of CDX2 (a), membranous staining of CD10 (c) and cytoplasmic expression of MUC2 in $>10\%$ of the tumor cells (e). Conversely, MUC5AC (g) and MUC6 (i) are negative in the tumor cells. An example of the gastric type shows staining of MUC5AC (h) and MUC6 (j) in the majority of the tumor cells, but they are not reactive to CDX2 (b), CD10 (d) or MUC2 (f).

Figure 2 Continued.

mucosa-associated adenocarcinomas were more likely to have nuclear β -catenin staining ($P=0.0080$), and less likely to exhibit *EGFR* amplification ($P=0.0051$). Even when tumors greater than 3 cm alone were compared between the two groups, the differences in immunophenotypes and biomarker expression were preserved (Table 3).

Clinicopathological Characteristics of Intestinal-Type Mucosa-Associated Adenocarcinomas by Surveillance History

To account for the effects of endoscopic surveillance on stage and outcome,^{1,17,38} we compared the clinicopathological characteristics and survival between intestinal-type mucosa-associated adenocarcinomas found during surveillance and outside surveillance (Table 4). In our intestinal-type mucosa-associated adenocarcinoma cohort, there were 30 surveillance cases and 78 cases diagnosed with a symptomatic carcinoma. Those with an invasive carcinoma found during surveillance were more likely to have reflux symptoms ($P=0.0071$), and were less likely to be anemic ($P=0.0093$).

Furthermore, surveillance cases were less likely to have tumors greater than 1 cm ($P=0.0001$), advanced T stage (T3 + T4, $P=0.021$) or MUC1 expression ($P=0.013$).

No significant differences among intestinal-type mucosa-associated adenocarcinomas existed between surveillance and symptomatic tumor groups in histomorphology, immunophenotype or nuclear expression of β -catenin. More importantly, there were no differences in common prognostic factors such as lymph node metastasis, lymphovascular invasion or margin status between the two groups.

Survival

Five-year overall survival rate for patients with intestinal-type mucosa-associated adenocarcinoma was 28% and that for cardiac-type mucosa-associated adenocarcinoma was 9% (log-rank $P=0.0015$). When patients with intestinal-type mucosa-associated adenocarcinoma were stratified based on a history of surveillance for Barrett esophagus, patients on surveillance experienced an overall survival similar to patients without

surveillance (log-rank $P=0.82$). Even stage by stage comparison did not reveal difference in overall survival between the two groups, although the low patient number in each category may have hampered statistical analysis.

Predictors of Survival

Using the Cox regression model, patients with intestinal-type mucosa-associated adenocarcinoma had a significant reduction in unadjusted overall

Figure 3 Mixed intestinal and gastric (a, c, e, g, i) and null (b, d, f, h, j) immunophenotypes. An example of the mixed intestinal and gastric type exhibits concurrent expression of two intestinal markers (CDX2 (a) and MUC2 (e)) and gastric markers (MUC5AC (g) and MUC6 (i)). This example is negative for CD10 (c). The null type is characterized by negative expression of all five markers: CDX2 (b), CD10 (d), MUC2 (f), MUC5AC (h), and MUC6 (j).

Figure 3 Continued.

Table 1 Clinicopathological characteristics by Siewert types

	Type 1 (n = 51)	Type 2 (n = 106)
Age at presentation (mean ± s.d.)	66 ± 11	65 ± 11
Male:female ratio*	46:5	82:24
Prevalence of intestinal metaplasia (%)**	86	60
Histology (A/G/P/Med/M/S) ^a	1/23/11/2/4/10	3/54/26/3/3/17
Immunophenotype (I/G/M/N) ^{b,c,***}	16/11/18/2	22/30/19/19
CDX2 (%) ^b	53	38

^aA: adenosquamous; G: low-grade conventional adenocarcinoma; P: high-grade conventional adenocarcinoma; Med: medullary carcinoma; M: mucinous carcinoma; S: signet ring cell carcinoma.

^bImmunostains were evaluated in 47 Siewert type 1 and 90 Siewert type 2 cases.

^cI: intestinal; G: gastric; M: mixed; N: null immunophenotype.

*P = 0.052; **P = 0.0009; ***P = 0.0037.

mortality compared with those with cardiac-type mucosa-associated adenocarcinoma (HR: 0.48; 95% CI: 0.30–0.76). However, after stratification by AJCC

tumor stage and adjustment for all the potential confounders, intestinal-type mucosa-associated adenocarcinomas showed no overall survival benefit (HR: 1.03; 95% CI: 0.62–1.71 and HR: 1.34; 95% CI 0.72–2.50, respectively). In this stage-matched Cox proportional hazard model, predictors of poor outcome included older age, the application of thoraco-abdominal or transhiatal approach, perineural invasion, tumor size >3 cm and MUC1 expression, whereas female gender, 1 cm or greater surgical clearance, and nuclear expression of β -catenin predicted a favorable outcome (Table 5). Similarly, upon multivariate analysis, there was no difference in overall mortality between patients with intestinal-type mucosa-associated adenocarcinoma and a history of surveillance vs those with intestinal-type mucosa-associated adenocarcinoma and no surveillance.

Discussion

Significant differences were found in mucin phenotype and molecular alterations between adenocarci-

Table 2 Clinical and tumor characteristics by the presence of adjacent intestinal metaplasia

	Intestinal metaplasia present (n = 108)	Intestinal metaplasia absent (n = 49)	P-value
Age (mean $\pm$ s.d.)	64 $\pm$ 11.1	67.3 $\pm$ 10.4	0.0057
Male:female ratio	91:17	37:12	0.19
History of surveillance (%)	28	0	<0.0001
Reflux (%)	67	28	<0.0001
Anemia (%)	40	33	0.39
GI bleed (%)	16	16	1.00
Dysphagia (%)	51	74	0.011
Location of tumor (Siewert type 1 vs type 2)	44/64	7/42	0.0009
Surgical procedures (IL/TA/TH) ^a	54/34/20	16/32/1	0.043 ^b
Size (cm, mean $\pm$ s.d.)	3.4 $\pm$ 2.0	4.9 $\pm$ 2.2	<0.0001
Tumors >1 cm (%)	86	100	0.0051
Tumors >3 cm (%)	54	86	0.0001
pT stage (1/2/3/4)	56/16/36/0	2/4/42/1	<0.0001 ^c
pN new AJCC (0/1/2/3)	64/20/12/10	8/9/18/14	0.0001 ^d
pM (Mx/M1)	105/3	47/2	0.50
Lymphovascular invasion (%)	46	82	<0.0001
Venous invasion (%)	19	41	0.0060
Perineural invasion (%)	34	73	<0.0001
Resection margin <1 cm (%)	69	92	0.0022
Resection margin positive (%)	9	27	0.0046
pG grade (1/2/3)	21/45/41	2/17/30	0.0095 ^e
Histology (A/G/P/	2/61/22/	2/16/15/	0.0062 ^f
Med/M/S) ^g	3/5/15	2/2/12	
Immunophenotype (I/G/M/N) ^{h,i}	26/25/31/10	12/16/6/11	0.015 ^j
CDX2 (%) ⁱ	49	28	0.020
MUC1 (%) ⁱ	61	57	0.59
β -Catenin (%) ⁱ	46	22	0.0080
FISH (<i>EGFR/ERBB2</i> /no amp)	5/9/94	9/2/38	0.0051 ^k

^aIL: Ivor–Lewis; TA: thoraco-abdominal; TH: transhiatal esophagectomy or esophagogastrectomy.

^bIL vs TA+TH.

^cT1–2 vs T3–4.

^dpN0 vs pN1–3.

^epG1–2 vs pG3.

^fA: adenosquamous; G: low-grade conventional adenocarcinoma; P: high-grade conventional adenocarcinoma; Med: medullary carcinoma; M: mucinous carcinoma; S: signet ring cell carcinoma.

^gG vs the others.

^hI: intestinal; G: gastric; M: mixed; N: null immunophenotype.

ⁱImmunostains were evaluated in 92 with and 45 without intestinal metaplasia cases.

^jI+M vs G+N.

^k*EGFR* positive vs *EGFR* negative.

nomas arising in association with Barrett mucosa and those that do not. Intestinal-type mucosa-associated adenocarcinomas were more likely to exhibit an intestinal or mixed intestinal/gastric immunophenotype, and express nuclear β -catenin. In contrast, cardiac-type mucosa-associated adenocarcinomas were more likely to correlate with *EGFR* amplification. These findings support the notion that two different pathways lead to adenocarcinomas of the distal esophagus and esophagogastric junction: one in which intestinal-type epithelium with goblet

Table 3 Clinical and tumor characteristics of large tumors (>3 cm)

	Intestinal metaplasia present (n = 56)	Intestinal metaplasia absent (n = 42)	P-value
Age (mean $\pm$ s.d.)	66.4 $\pm$ 10.7	66.2 $\pm$ 10.7	0.91
Male:female ratio	50:6	30:12	0.034
History of surveillance (%)	16	0	0.0091
Reflux (%)	37	0	<0.0001
Anemia (%)	50	31	0.066
GI bleed (%)	16	18	0.78
Dysphagia (%)	59	75	0.17
Location of tumor (Siewert type 1 vs type 2)	26/30	6/36	0.0010
Surgical procedures (IL/TA/TH) ^a	27/17/12	15/26/1	0.30 ^b
Size (cm, mean $\pm$ s.d.)	4.9 $\pm$ 1.5	5.3 $\pm$ 2.1	0.24
pT stage (1/2/3/4)	14/12/30/0	2/2/37/1	0.0001 ^c
pN new AJCC (0/1/2/3)	26/12/8/10	7/6/17/12	0.0024 ^d
PM (Mx/M1)	53/3	40/2	1.00
Lymphovascular invasion (%)	59	83	0.013
Venous invasion (%)	29	40	0.37
Perineural invasion (%)	45	76	0.0035
Resection margin <1 cm (%)	84	93	0.23
Resection margin positive (%)	16	29	0.15
pG grade (1/2/3)	2/25/29	0/16/26	0.41 ^e
Histology (A/G/P/Med/M/S) ^f	1/23/15/3/5/9	2/14/11/2/7/6	0.53 ^g
Immunophenotype (I/G/M/N) ^{h,i}	16/12/18/6	12/13/5/9	0.038 ^j
CDX2 (%) ⁱ	54	30	0.033
MUC1 (%) ⁱ	71	55	0.13
β -Catenin (%) ⁱ	52	23	0.0089
FISH (<i>EGFR/ERBB2</i> /no amp)	2/5/49	7/2/33	0.033 ^k

^aIL: Ivor–Lewis; TA: thoraco-abdominal; TH: transhiatal esophagectomy or esophagogastrectomy.

^bIL vs TA+TH.

^cT1–2 vs T3–4.

^dpN0 vs pN1–3.

^epG1–2 vs pG3.

^fA: adenosquamous; G: low-grade conventional adenocarcinoma; P: high-grade conventional adenocarcinoma; Med: medullary carcinoma; M: mucinous carcinoma; S: signet ring cell carcinoma.

^gG vs the others.

^hI: intestinal; G: gastric; M: mixed; N: null immunophenotype.

ⁱImmunostains were evaluated in 52 cases with and 39 without intestinal metaplasia.

^jI+M vs G+N.

^k*EGFR* positive vs *EGFR* negative.

cells becomes dysplastic (intestinal pathway) and another in which dysplasia arises in cardiac-type glandular mucosa (non-intestinal pathway).^{39–41} In contrast, an alternative explanation for the lack of intestinal metaplasia in some adenocarcinomas,³⁹ overgrowth of short segment intestinal metaplasia by large tumors,^{17,42} explains neither the absence of intestinal-type mucosa in certain small esophageal adenocarcinomas⁴³ nor our observation of phenotypic and molecular differences. Thus, adenocarcinomas of the distal esophagus and esophagogastric junction may be stratified based on the presence or absence of background Barrett mucosa, with unique clinicopathological characteristics in either group, but without significant survival differences.

Table 4 Clinical and tumor characteristics of intestinal-type mucosa-associated adenocarcinoma stratified by history of surveillance

	No surveillance (n = 78)	Surveillance (n = 30)	P-value
Age (mean $\pm$ s.d.)	65.9 $\pm$ 11.2	60.6 $\pm$ 9.9	0.025
Male:female ratio	65:13	26:4	0.77
Reflux (%)	59	89	0.0071
Anemia (%)	47	20	0.0093
GI bleed (%)	20	4	0.10
Dysphagia (%)	53	44	0.45
Location of tumor (Siewert type 1 vs type 2)	33/45	11/19	0.67
Surgical procedures (IL/TA/TH) ^a	38/26/14	16/8/6	0.80 ^b
Size (cm, mean $\pm$ s.d.)	3.9 $\pm$ 2.0	2.0 $\pm$ 1.3	<0.0001
Tumors > 1 cm (%)	95	63	0.0001
Tumors > 3 cm (%)	64	30	0.0024
pT stage (1/2/3/4)	35/11/32/0	21/5/4/0	0.021 ^c
pN new AJCC (0/1/2/3)	42/15/9/10	22/5/3/0	0.14 ^d
pM (Mx/M1)	75/3	30/0	0.56
Lymphovascular invasion (%)	50	35	0.18
Venous invasion (%)	23	8	0.14
Perineural invasion (%)	37	26	0.33
Resection margin < 1 cm (%)	74	57	0.10
Resection margin positive (%)	10	7	0.72
pG grade (1/2/3)	10/38/30	12/7/11	1.00 ^e
Histology (A/G/P/Med/M/S) ^f	2/43/13/3/4/13	0/18/9/0/1/2	0.67 ^g
Immunophenotype (I/G/M/N) ^{h,i}	20/20/25/7	6/5/6/3	0.84 ^j
CDX2 (%) ^j	49	48	0.89
MUC1 (%) ^j	68	38	0.013
β -Catenin (%) ^j	48	37	0.39
FISH (<i>EGFR/ERBB2</i> /no amp)	3/7/68	2/2/26	0.62 ^k

^aIL: Ivor–Lewis; TA: thoraco-abdominal; TH: transhiatal esophagectomy or esophagogastrectomy.

^bIL vs TA+TH.

^cT1–2 vs T3–4.

^dpN0 vs pN1–3.

^epG1–2 vs 3.

^fA: adenosquamous; G: low-grade conventional adenocarcinoma; P: high-grade conventional adenocarcinoma; Med: medullary carcinoma; M: mucinous carcinoma; S: signet ring cell carcinoma.

^gG vs the others.

^hI: intestinal; G: gastric; M: mixed; N: null immunophenotype.

ⁱImmunostains were evaluated in 20 cases with and 72 without surveillance.

^jI+M vs G+N.

^k*EGFR* positive vs *EGFR* negative.

The association of an intestinal or mixed immunophenotype (especially CDX2 expression) with intestinal-type mucosa-associated adenocarcinoma is in keeping with current understanding of the pathogenesis of intestinal metaplasia. Normally CDX2 is localized to the small and large bowel,^{44,45} where it is responsible for the maintenance of intestinal phenotype,^{21,22} including the upregulation of MUC2, an intestinal-type mucin, in goblet cells.^{31,46} CDX2 expression has also been reported in 54% of gastric carcinomas, often in conjunction with MUC2 expression.⁴⁷ In this milieu, CDX2 is associated with tumor progression, during which tumors undergo an immunophenotypic shift from gastric phenotype (MUC5AC and/or MUC6 positive) to intestinal phenotype.³⁰ Thus, intestinal-type differentiation can be seen in both pre- and post-neoplastic transformation. Conversely, loss or decrease in CDX2 expression has been shown in Barrett esophagus-related dysplasia.^{48,49} This observation raises the possibility that the association of intestinal-type mucosa-associated adenocarcinoma with an intestinal or mixed phenotype merely reflects the fact that it presents at a lower tumor stage than cardiac-type mucosa-associated adenocarcinoma. However, in our population, the difference in the prevalence of the intestinal and mixed phenotype between intestinal- or cardiac-type mucosa-associated adenocarcinomas was preserved even in large tumors (>3 cm in diameter), and in more advanced stage tumors.

β -Catenin is normally expressed on the cell membrane in non-dysplastic esophageal squamous epithelium and Barrett esophagus.⁵⁰ Abnormal expression (loss of membranous expression and focal nuclear staining) can be seen even in low-grade dysplasia and is prevalent in carcinoma.^{50–52} Thus, nuclear accumulation of β -catenin is considered to be a common and early event during neoplastic progression of Barrett esophagus.⁵³ Abnormal β -catenin expression has also been reported in gastric adenocarcinoma, especially in association with intestinal phenotype, although it is usually seen in more advanced tumors.²⁷ In our study, we found that abnormal nuclear β -catenin expression was more prevalent in intestinal-type mucosa-associated adenocarcinoma than in cardiac-type mucosa-associated adenocarcinoma, confirming that β -catenin misregulation may have a specific role in carcinogenesis of the intestinal-type mucosa. Nuclear expression of β -catenin was also associated with favorable outcomes in multivariate analysis. This is in keeping with a previous report that abnormal β -catenin expression was associated with better survival irrespective of depth of invasion in patients with intestinal-type mucosa-associated adenocarcinoma.²⁹

Increased expression of *EGFR* and/or amplification of 7p12 (chromosomal locus of *EGFR*) have been reported in 30–60% of esophageal and esophagogastric junction adenocarcinomas.^{32,33,54}

Table 5 Multivariate analysis of factors associated with survival after resection

	P-value	HR	95% CI
Age	<0.0001	1.07	1.04–1.10
Gender (female)	0.037	0.40	0.17–0.95
Anemia	0.018	1.85	1.04–3.28
Surgical procedure (TA or TH)	0.035	2.14	1.13–4.06
Perineural invasion	0.0004	3.88	1.83–8.21
Tumor size > 3 cm	0.0084	2.75	1.30–5.82
Surgical clearance > 1 cm	0.037	0.39	0.16–0.94
β -Catenin (nuclear)	0.026	0.48	0.25–0.92
MUC1	0.019	2.14	1.13–4.06
Barrett esophagus	0.36		
Reflux symptom	0.19		

Interestingly, while elevated levels of *EGFR* have been identified in non-dysplastic intestinal metaplasia,^{55–57} we found *EGFR* amplification to be more highly associated with cardiac-type mucosa-associated adenocarcinoma. *EGFR* has also been linked to high-grade tumors and poor survival.^{32–34} Given these findings, *EGFR* amplification could be construed to be a later event in more advanced cancers. However, even when comparing only large tumors, *EGFR* amplification was significantly more frequent in cardiac-type mucosa-associated adenocarcinomas than in intestinal-type mucosa-associated adenocarcinomas. Taken together with the other immunophenotypic differences between intestinal- and cardiac-type mucosa-associated adenocarcinomas, this suggests that different pathways are involved in carcinogenesis of the two types of adenocarcinoma arising in the vicinity of the esophagogastric junction.

Growing evidence has also suggested the presence of a non-intestinal-type neoplastic pathway in the distal esophagus and esophagogastric junction. In one study of 300 patients older than 65 years, 36% showed endoscopic evidence of short segment Barrett esophagus. Of those, the majority (57%) did not exhibit microscopic intestinal metaplasia.⁵⁸ In another large cohort, 45% of those with endoscopically diagnosed Barrett esophagus had no microscopic evidence of intestinal metaplasia, yet 3.6% ultimately developed adenocarcinoma. This was not statistically different from the 7.4% of patients with Barrett esophagus who developed adenocarcinoma.⁵⁹ On the basis of these results, it is reasonable to speculate that metaplastic columnar epithelium without intestinal metaplasia (gastric/cardiac type metaplasia) in the distal esophagus and esophagogastric junction is not uncommon, especially in the older population, and could be a precursor to the non-intestinal-type adenocarcinoma. Therefore, our results, if confirmed, would support the recent recommendation of AGA that defines 'Barrett esophagus' as the condition in which any extent of metaplastic columnar epithelium (irrespective of the presence of goblet cells) that predisposes to cancer development replaces the native squamous epithelium of the distal esophagus,⁶⁰ and could be the basis for challenging the current surveillance protocol.

Although our results revealed the difference in clinicopathological characteristics between intestinal- and cardiac-type mucosa-associated adenocarcinomas, we did not find a survival advantage by either group, after stratification by tumor stage. Instead, survival was affected by other well-known prognostic factors, including perineural invasion, tumor size, age and gender.⁶¹ Although stage for stage, the presence of intestinal metaplasia did not affect tumor prognosis, intestinal-type mucosa-associated adenocarcinoma often presented at an earlier stage. This could be attributed to either endoscopic surveillance or symptomatic gastroesophageal

reflux,⁶² prompting an endoscopic workup with incidental identification of malignancy. The results of this study, however, raise the possibility of well-established intestinal milieu preventing tumor progression. In support of this hypothesis, it has been reported that CDX2 has a tumor suppressor function in the colon.⁶³ *Cdx2*^{+/-} mice developed adenocarcinomas after carcinogen treatment specifically in the distal colon, where the lowest expression of *Cdx2* is found.⁶³ Thus, lack or reduced CDX2 of cardiac-type epithelium compared with intestinal-type epithelium in the distal esophagus and esophagogastric junction⁴⁸ may facilitate tumor progression. This hypothesis, however, has yet to be confirmed in prospective studies.

Our study has several limitations that are inherent to a retrospective study using resected specimens. Most importantly, the absence of intestinal metaplasia in non-neoplastic columnar mucosa could be attributed to incomplete sampling of the lesion, especially in large tumors that involved the esophagogastric junction circumferentially. Such tumors may have overgrown short segment Barrett esophagus as well.^{17,42} In our cohort, 24% (20/83) of the patients with a preoperative diagnosis of pathologically confirmed Barrett esophagus failed to reveal intestinal metaplasia of the esophagus in the examined sections (data not shown). To overcome this issue, we included such cases in the intestinal-type mucosa-associated adenocarcinoma group, and subsequently showed the difference in clinical characteristics, such as the presence of reflux symptoms, between intestinal- and cardiac-type mucosa-associated adenocarcinoma cases. Furthermore, the difference in clinicopathological characteristics between the two groups was preserved even when large tumors alone were compared. To accurately assess tumor immunophenotype and histomorphological characteristics, we excluded a large group of tumors with preoperative therapy. The exclusion of these cases resulted in a relatively small study size, and it is possible that the lack of statistical significance in some of our analyses is due to inadequate case numbers. A larger study might be helpful in this regard.

In summary, review of adenocarcinomas of the distal esophagus and esophagogastric junction at our institution found that tumors arising in association with Barrett esophagus had significantly different immunophenotypic and molecular characteristics from those arising in cardiac-type mucosa, supporting the theory that divergent pathways of metaplasia and dysplasia exist in esophagogastric tumorigenesis. Additional studies are warranted to further clarify their pathogenesis and the molecular mechanisms involved.

Disclosure/conflict of interest

The authors declare no conflict of interest.

Author contributions: Study concept and design: Mari Mino-Kenudson; acquisition of data: Elizabeth G Demicco, Alton B Farris III, Brian Agbor-ETang, Kristin Bergethon, Rajini Mandal, Diane Davies, Junya Fukuoka, Michio Shiizu, Dora Dias-Santagata, and Mari Mino-Kenudson; analysis and interpretation: Elizabeth G Demicco, A John Iafrate, and Mari Mino-Kenudson; drafting of the manuscript: Elizabeth G Demicco, Henning A Gaisert, and Mari Mino-Kenudson; critical revision of the manuscript for important intellectual content: Elizabeth G Demicco, Alton B Farris III, Yoshifumi Baba, Brian Agbor-ETang, Kristin Bergethon, Rajini Mandal, Diane Davies, Junya Fukuoka, Michio Shiizu, Dora Dias-Santagata, Shuji Ogino, A John Iafrate, Hennig A Gaisert, and Mari Mino-Kenudson; statistical analysis: Yoshifumi Baba and Shuji Ogino; study supervision: Henning A Gaisert and Mari Mino-Kenudson.

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