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MUC2 Expression is an Adverse Prognostic Factor in Superficial Gastroesophageal Adenocarcinomas

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Abstract

Mucin core proteins (MUCs) are expressed in tissue specific patterns in the gastrointestinal tract and expression is deregulated in Barrett's metaplasia. Based on differential expression, MUCs have been used to classify adenocarcinomas into distinct phenotypes (e.g. intestinal, gastric, pancreaticobiliary, etc). Because MUC expression patterns carry prognostic significance in other tumors, we evaluated MUC expression in superficial adenocarcinomas of the gastroesophageal junction and esophagus (EAC) to determine whether there are differences in outcome associated with MUC subtype in this potentially curable subset of EAC. We classified 142 resected, superficial (T1) EAC based on their pattern of expression of MUC2, MUC5AC, MUC6 and MUC1. The association between survival and MUC expression pattern was determined in univariate and multivariate analyses. The MUC2 positive "intestinal" phenotype was associated with significantly worse prognosis in submucosal EAC (HR 2.2, 95%CI 1.2–4.2), independent of node stage and other prognostic factors. MUC2 expression in submucosal EAC also showed significantly accelerated time to recurrence (HR 2.8, 95% CI 1.2–6.8) after adjusting for node stage. The classification of superficial EAC by MUC protein expression has prognostic significance. MUC2 expression is an adverse prognostic indicator in submucosal EAC, independent of node stage and other prognostic factors.

Keywords

MUC2 protein; esophagus; mucins; survival; glandular and epithelial neoplasms

Introduction

The mucin core proteins (MUCs) are glycosylated proteins expressed in tissue specific patterns in the gastrointestinal tract, including MUC1 and MUC5AC in the gastric foveolar epithelium, MUC6 in the pyloric-type glandular epithelium and MUC2 in intestinal goblet

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cells (1). In the context of esophageal disease, all of these MUC subtypes are aberrantly expressed in Barrett's metaplasia (2–4). Furthermore, changes such as downregulation of MUC2, MUC5AC, and MUC6 and upregulation of MUC1 have been described in the neoplastic progression from Barrett's esophagus to dysplasia and adenocarcinoma (2, 5) and adenocarcinomas of the distal esophagus and gastroesophageal junction more frequently express markers of intestinal differentiation (including MUC2) if they arise in a background of intestinal metaplasia (6).

Because of their restricted range of expression within normal tissues of the gastrointestinal tract, MUCs have been used as differentiation markers to classify adenocarcinomas into intestinal, gastric and pancreaticobiliary subtypes. Beyond this purely descriptive role in gastrointestinal and pancreaticobiliary tumor classification, MUC-based categorization can stratify tumors into prognostically distinct subgroups (7–9). Most recently, the utility of immunophenotypic subtyping was demonstrated for ampullary adenocarcinomas, for which the pancreaticobiliary phenotype had a negative prognostic association in a training cohort and 2 independent validation cohorts (9).

Superficially invasive gastroesophageal adenocarcinomas (EAC), by definition, invade no deeper than the submucosa. Superficial EAC are increasingly prevalent, likely because of screening for and surveillance of Barrett's esophagus, and have a favorable prognosis relative to more deeply invasive EACs (10). This favorable prognosis has prompted a recent paradigm shift away from esophagectomy toward esophagus-preserving treatment approaches such as endoscopic mucosal resection and avoidance of neoadjuvant and adjuvant chemoradiotherapy for superficial tumors due to the low likelihood of nodal metastasis (11). In this setting, where multiple therapeutic options are available and opportunities for effective systemic therapy may be missed, prognostic biomarkers are needed to optimize decisions for individual patients. A three-gene immunohistochemical biomarker panel (*EGFR*, *SIRT2* and *TRIM44*) was recently reported to stratify EAC into prognostic subgroups independent of pathologic stage. But the three marker panel performed best in Stage II and Stage III subgroups (12), underscoring the need for studies focused on clinically distinct subgroups of patients such as superficial EAC.

In this report, we hypothesized that MUC expression patterns could be used to classify superficial EAC into prognostically distinct subtypes because of the association of epithelial differentiation marker expression with prognosis in other tumors. To test this hypothesis, we determined the clinical and histopathologic correlates of the MUC immunophenotype and evaluated the prognostic significance of the classification scheme. Because they are potential regulators of MUC gene expression, we also correlated MUC expression with CDX2 expression and *EGFR* gene amplification status (13, 14).

Methods

Case Selection

We searched the institutional pathology archive for superficially invasive adenocarcinoma (tumor classification T1) of the distal esophagus or gastroesophageal junction treated by esophagectomy at our institution between 1996 and 2010. We excluded patients with high grade dysplasia only, neo-adjuvant therapy, carcinoma limited to the proximal stomach, squamous cell carcinoma and adenocarcinoma invading beyond the submucosa (tumor classification T2 or higher). We identified 142 cases with archival tissue blocks available for inclusion in a tissue microarray. This study protocol was approved by our Institutional Review Board.

Clinicopathologic Variable Definitions

TNM classifications and stage groupings were based on the 7th Edition of the American Joint Committee on Cancer (AJCC) Staging Manual for EAC (15). Barrett's esophagus (intestinal metaplasia of the esophagus) was present if it was diagnosed in a pre-operative endoscopy with biopsy confirmation or if it was identified histologically in the esophagectomy specimen. Tumor location was assigned on the basis of retrospective review of the gross description and available digital images of the resection specimen and classified as GE junction or esophageal based on AJCC criteria (15), tumors were classified as esophageal if they were located in the esophagus and did not cross the gastroesophageal junction as defined by the upper extent of the gastric folds. Tumors were classified as gastroesophageal junction if they were centered in the proximal 5 cm of the stomach or distal 5 cm of the esophagus and invaded across the gastroesophageal junction. Tumors centered in the proximal stomach that did not invade into the esophagus were not included in the study. Clinical data, including data used to calculate the Charlson comorbidity index (16), were abstracted from the patients' medical record. Survival data were obtained from the patient's medical record and the social security death index. Overall survival was calculated from the time of esophagectomy to the date of death and censored at the last date the patient was confirmed to be alive. Time to recurrence was calculated from the date of esophagectomy to the date of first recurrence and censored at the most recent negative clinical evaluation for recurrence. Recurrences were classified as locoregional (anastomotic or regional lymph nodes) and distant (liver, lung, bone, etc.). Patients with treatment related mortality (< 3 months survival) were excluded from the survival analyses (n=9).

Pathology Review

Representative tumor slides were reviewed to confirm superficial invasion and identify WHO histopathologic subtypes (17). Tumors were classified as colloid, signet ring or diffuse type if one of these patterns was present in >10% of the tumor. The remainder could all be classified as tubulopapillary. There were 2 cases in which >10% of the tumor was colloid and >10% was signet ring type. In these cases, the tumor was classified based on the predominant pattern (1 of each). All other pathologic data were abstracted from the original pathology report.

Tissue Microarray (TMA) Construction

Representative areas of the carcinoma were targeted with up to three 0.6 mm cores using a Beecher Instruments (Sun Prairie, WI) manual arrayer. The arrays were sectioned at 5 micron intervals with 1 H&E stained section every 50 microns.

Immunohistochemistry

Immunohistochemistry was performed as described previously (18) with the following mouse monoclonal antibodies: MUC1 (Ma552, 1:100, Novocastra, Newcastle Upon Tyne, UK), MUC2 (Ccp58, 1:100, Abcam, Cambridge, UK), MUC5AC (CLH2, 1:100, Vector, Burlingame, CA), MUC6 (CLH5, 1:100, Vector), CDX2 (CDX2-88, 1:100, Biogenex, Fremont, CA). Briefly, 4 µm paraffin sections were deparaffinized in xylene solutions and rehydrated in graded alcohol solutions followed by washes in distilled water. Antigen retrieval was performed in a pressure cooker for 15 minutes in 10 mM citrate buffer (pH 6.0). The sections were allowed to cool to room temperature and then washed with PBS, 0.05% Tween 20, pH 7.0. The sections were incubated overnight in a humidified chamber at room temperature with the primary antibody. The Dako Envision system with a DAB chromogen was used for detection of the primary antibody, followed by hematoxylin counterstain.

To provide a semi-quantitative description of the extent of MUC and CDX2 immunoreactivity in each tumor, we used a modified H score (19). Cores were scored for intensity of staining in tumor epithelial cells (0, no staining; 1, weak staining; 2, moderate to strong staining) and the percentage of positive cells (illustrated in Figure 1). The H score is the product of the intensity and percentage of tumor epithelial cells with positive staining and yields possible scores between 0 and 200. In order to minimize false negative scores, a case was considered positive if any core had an H score >10 (e.g. moderate to strong staining in >5% of tumor cells). This definition was used for all analyses unless otherwise stated. All immunostains were scored based on the consensus of two independent scorers (JMD and either SE or TF). Scoring of immunostains was performed while blinded to other variables.

Fluorescence in Situ Hybridization

TMA sections were hybridized with *EGFR* SpectrumOrange and centromere 7 (CEP7) SpectrumGreen probes (Abbott Molecular, DesPlaines, IL) as described previously (20). Each case was scored by counting 30 individual tumor nuclei and calculating the average *EGFR* signal to CEP7 signal count per nucleus (*EGFR*/CEP7). We used a cutoff of *EGFR*/CEP7 ≥ 2 for amplification (21).

Whole Section Immunohistochemistry for Validation of TMA Scores

Whole sections cut from 26 donor paraffin blocks containing tumor were stained with the MUC2 antibody in order to validate the TMA score. Whole sections were scored blinded to the TMA score. The invasive carcinoma was scored positive if there was staining in >5% of tumor cells. Cases with rare, isolated tumor cells expressing MUC2 were considered negative.

Statistical Analysis

Differences in categorical variables were assessed by chi-squared or two-sided Fisher's exact test as appropriate. Kaplan-Meier survival functions were used to plot overall survival and differences in survival functions were evaluated by log rank test. A multivariate Cox proportional hazards model was constructed using variables analyzed in univariate Cox models, employing an *a priori* cutoff of $p < 0.05$ for inclusion. Calculations were performed with SPSS software, version 20 (IBM Corporation, Armonk, NY).

Results

Patterns of Mucin Protein Expression in EAC

MUC1 was positive in the large majority (119 cases, 83%) of superficial EAC. The other gastric MUCs (MUC5AC and MUC6) were positive in 106 cases (74%) and 100 cases (70%), respectively. MUC2 was expressed in only 57 cases (40%).

Tumors that express MUC2 were classified as "intestinal" type because MUC2 is normally expressed in intestinal goblet cells. However, 55/57 (97%) of MUC2 positive tumors showed a mixed gastro-intestinal phenotype due to co-expression of gastric MUC antigens.

There were 84 tumors that had a "gastric" phenotype (MUC2 negative) and 67/84 (80%) expressed more than one gastric antigen. Of the 17 tumors that only expressed one gastric MUC antigen, 8/17 expressed only MUC1, 7/17 cases expressed only MUC6 and 2/17 cases only expressed MUC5AC. Two cases lacked detectable expression of the four MUC proteins.

Qualitatively, cases that we scored as negative had almost entirely absent staining in all cores. Positive MUC staining was more heterogeneous and variable. High protein expression

(defined as H score > 100; i.e. staining in >50% of cells) was seen in a minority of cases with positive expression, as follows: MUC5AC, 49/116 (46%); MUC2, 15/57 (26%); MUC6, 19/100 (19%); and MUC1, 24/119 (20%).

Clinical and Pathologic Characteristics of the Patient Population

The clinical and pathologic features of our study population are summarized in Table 1. The majority (81%) of patients were treated by minimally invasive esophagectomy, a combined laparoscopic and thoracoscopic procedure, with extensive lymph node dissection (22). Most of the remaining patients underwent an open transhiatal esophagectomy.

Relationship of Mucin Phenotype to Histologic type, CDX2 Expression and EGFR Amplification

Based on a previous report, MUC2 expression is expected in adenocarcinomas with mucinous histology (23). Ten of 11 (91%) superficial EAC with at least a minor mucinous (colloid) component were MUC2 positive (Table 2, Figure 2). Only 4 cases met WHO criteria for mucinous (colloid) adenocarcinoma (>50% of the tumor exhibiting extracellular mucin production) and all 4 expressed MUC2. The others with mucinous differentiation only had a minor (10–50%) mucinous component. The one signet ring cell adenocarcinoma (comprised of >50% signet ring cells) also expressed MUC2. All four tumors with >10% diffuse type carcinoma were gastric type.

We looked at the correlation of MUC expression with CDX2 and *EGFR*, two potential regulators of MUC gene expression (13, 14). Similar to MUC expression, CDX2 expression was heterogeneous, but showed high expression levels (H score > 100; i.e. staining in at least 50% of tumor nuclei) more commonly in intestinal type EAC (Table 2, Figure 2). We also looked for an association between MUC2 expression and *EGFR* amplification because not only has *EGFR* been shown to regulate MUC2 expression in colon cancer cells (14), but high level *EGFR* gene amplification (*EGFR*/CEP7 15) may be a poor prognostic factor in EAC (21). High level amplification was identified in only 4 of 125 (3%) superficial EACs and there was no association between *EGFR* amplification (low or high level) and mucin phenotype (Table 2).

MUC2 Expression (Intestinal Type) is Associated with Poor Prognosis in Submucosal (T1b) EAC

There were 49 deaths in the analyzed cohort and the 3 and 5 year overall survival rates were 69% and 58%. When we compared survival of MUC2-positive intestinal type cancers with MUC2-negative gastric type cancers there was a survival trend favoring the gastric type, but the trend was not significant in the overall cohort (74% vs. 60% alive respectively at 36 months, $p = 0.092$, log rank test). When we excluded intramucosal EAC, we found that submucosal (T1b) EAC with an intestinal phenotype had significantly worse overall survival than gastric or ductal type T1b EAC (Figure 3A). There was no association between intestinal MUC phenotype and survival in intramucosal (T1a) EAC ($p = 0.966$, log rank test).

In a multivariate Cox proportional hazards analysis of survival in patients with submucosally invasive (T1b) EAC, the intestinal phenotype was associated with a 2.2-fold (95% confidence interval (CI)=1.2–4.2) increase in the risk of death, independent of other potentially confounding prognostic variables, including the presence of nodal metastasis (Table 3).

There is conflicting data in the literature concerning the prognostic significance of the mucinous histologic subtype of EAC (colloid or signet ring cell) (24, 25). We repeated the

multivariate survival analysis after excluding 5 cases that met the WHO definition of mucinous (n=4) or signet ring cell (n=1) adenocarcinoma and found that MUC2 expression (intestinal phenotype) remained a significant, independent prognostic factor in T1b EAC (hazard ratio=2.1, 95% CI=1.1–4.0) when controlling for the other prognostic variables in the multivariate analysis (tumor size, lymph node metastasis). We also separately examined the association between high MUC2 expression (H score>100) and survival in T1b EAC, but there were only 8 cases with high level MUC2 expression. There was a difference in survival (median survival of 26 months for high MUC2 versus 74 months for lower MUC2 expression) but it was not significant (p=0.310, log rank test).

MUC2 Expression (Intestinal Type) in Submucosal (T1b) EAC is Associated with Significantly Shorter Time to Recurrence

Time to recurrence is a superior indicator of aggressive tumor behavior in EAC than overall survival, particularly in a patient population with other comorbidities and tumors with relatively favorable prognosis. In the entire cohort of patients, there were 24 recurrences and 23 of 24 patients with tumor recurrence died during the follow up interval. The proportion of patients remaining recurrence free at 2 years was 81%. There were 13 recurrences at distant sites (e.g. liver, lung metastases), 3 at local/regional sites and 8 recurrences at both local/regional sites and distant sites.

However, intestinal type submucosal EAC had a significantly shorter time to recurrence than MUC2 negative gastric type (Figure 3B). There remained a 2.8-fold (95% CI 1.2–6.8) higher risk of recurrence for intestinal type submucosal EAC when controlling for nodal stage. The mucin phenotype was not predictive of recurrence in intramucosal (T1a) EAC.

MUC2 Expression is a prognostic factor in Node Negative Submucosal EAC

Because submucosal, node negative (pT1bN0) EAC are very unlikely to receive adjuvant chemotherapy in the immediate postoperative setting, we evaluated the prognostic significance of the intestinal phenotype in this specific subset. There were 74 T1bN0 cases (45 gastric type and 29 intestinal type). Relative to gastric type, the intestinal type had significantly reduced overall survival (47% versus 83% 3 year survival, p=0.005, log rank test) and lower likelihood of remaining recurrence free (57% versus 93% recurrence free at 3 years, p=0.002, log rank test).

MUC2 Scoring is Reproducible in Whole Tumor Sections

In order to assess the reproducibility of MUC2 scoring, we stained 26 cases representing a distribution of MUC2 expression by TMA. There was agreement between the MUC2 TMA score and the whole section score in 24 of 26 cases when whole sections were scored blinded to the TMA result, including 8 of 8 negative cases and 16 of 18 positive cases (kappa = 0.831). The two false negatives (scored negative on the TMA, but positive on whole sections) showed a low level of MUC2 expression on the whole tumor sections (<20% of cells), on review. Because EAC tends to lose expression of MUC2 during tumor progression, non-invasive dysplasia may express higher levels of MUC2 than the invasive carcinoma. We scored only the invasive component.

Discussion

In this study we evaluated the mucin core protein (MUC) expression pattern in 142 induction-therapy naïve, surgically resected, superficial (T1) EAC. Prior studies have illustrated that mucin core proteins are expressed in EAC (5, 26–29), but our study is the first to explore the prognostic significance of subclassifying superficial EAC based on the pattern of MUC protein expression.

Our central finding in this study is a significant, negative prognostic association with the intestinal phenotype in submucosally invasive EAC when controlling for potential confounding factors. The fact that we failed to detect an association of the MUC phenotype with survival in intramucosal (T1a) EAC is not surprising given the favorable prognosis associated with these very superficial lesions. The prognostic significance of subclassifying esophageal carcinomas (squamous cell carcinoma versus adenocarcinoma) is well established and is now integrated into the widely used 7th edition of the AJCC staging system for esophageal carcinoma (30). The potential utility of further subclassifying gastrointestinal adenocarcinomas based on expression of gastrointestinal differentiation markers has recently been highlighted by a large study of ampullary carcinomas that found a negative prognostic association with the pancreaticobiliary phenotype of ampullary adenocarcinoma (25).

A previous study of EAC that included a wider range tumor stages (6) evaluated the epithelial phenotype with MUC as well as non-MUC proteins (CD10 and CDX2) and did not report an association with survival for the intestinal phenotype. In more deeply invasive tumors, the epithelial immunophenotype may have less prognostic significance than other factors such as nodal metastasis as is true for the prognostic difference between squamous cell and adenocarcinoma (30) of the esophagus.

The mechanism by which MUC2 expression could influence the clinical behavior of a tumor has not been well studied. The association between MUC2 expression and poor survival may be an indirect consequence of signaling changes in the tumor cells that cause persistent MUC2 expression (31, 32). Our study provides some evidence of this possibility in the positive correlation between CDX2 expression and MUC2 expression. CDX2 is known to regulate MUC2 expression in both gastric cancer cells and colon cancer cells (13). We also evaluated the association of MUC2 expression with *EGFR* amplification as *EGFR* is known to be amplified in a clinically aggressive subset of EAC (21) and has been shown to regulate MUC2 expression in colon cancer cells (14). However, we saw no association between MUC2 expression and *EGFR* amplification. High-level *EGFR* amplification described by Marx et al. (21), was rare in our study, precluding a conclusive assessment of its influence on survival in superficial EAC.

Our study has limitations. First, the study is retrospective. Hence, patients did not receive standardized therapy or follow-up. We attempted to control for important variables such as pre-operative comorbidities, type of operative procedure, number of lymph nodes resected *et cetera*, but we do not know how adjuvant treatment may have influenced survival. Secondly, the MUC proteins are potentially useful, but not ideal markers because expression is heterogeneous—spanning a wide spectrum from focal positivity to extensive expression within the tumor—a feature that may complicate interpretation. We compensated for the heterogeneity of MUC staining by adopting a low cutoff for calling a tumor positive in an effort to avoid false negative classification errors and this showed very good correlation with whole sections for MUC2 expression. Lastly, our results should be validated in an independent cohort with careful attention to scoring cutoffs (33).

In summary, we have explored the prognostic significance of MUC expression patterns in superficial EAC. The MUC2 positive intestinal phenotype is associated with poor prognosis in submucosally invasive EAC when controlling for other known prognostic variables and specifically applies to patients with node negative disease (pT1bN0) who are unlikely to be considered for adjuvant therapy based solely on TNM stage. These findings suggest that this subclassification may have value in the risk assessment of superficial EAC in combination with established pathologic risk factors such as node status, depth of invasion, tumor grade, angiolymphatic invasion when determining appropriate therapy.

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Figure 1. Representative immunohistochemical staining of superficial EAC with antibodies to mucin core proteins (MUCs)

There is heterogeneous MUC1 staining in (A), but stronger, more uniform, cytoplasmic and membranous MUC1 staining in a poorly differentiated adenocarcinoma in (B). There is heterogeneous cytoplasmic staining with MUC2 illustrated in (C) and predominantly strong and uniform cytoplasmic MUC2 staining in (D). There is predominantly weak, heterogeneous MUC5AC staining in (E) and strong, uniform cytoplasmic staining for MUC5AC in (F). There is heterogeneous cytoplasmic MUC6 staining in (G) with strong and uniform cytoplasmic staining with MUC6 in (H). 200X magnification for all images.

Figure 2. Examples of MUC2 expression in mucinous (colloid) adenocarcinoma and CDX2-positive adenocarcinoma

(A) Adenocarcinoma with mucinous (colloid) differentiation (whole section, 40X magnification). (B) The same tumor (as depicted in A) expresses MUC2 (whole section, 100X magnification). (C) High level expression of CDX2 in a representative tumor core (100X magnification). (D) The same tumor was positive for MUC2 (100X magnification).

Figure 3. Kaplan-Meier survival functions for submucosal (T1b) EAC comparing mucin immunophenotypes

(A) Overall survival in submucosal EAC; (B) time to recurrence in submucosal EAC. In both analyses, the MUC2 positive intestinal phenotype is associated with significantly worse prognosis (log rank test). Patients with treatment related mortality (post-operative survival < 3 months) were excluded from the analysis (n=5).

Table 1

Clinical and Pathologic Features of the Patient Population Stratified by Mucin Phenotype

Clinical and Pathologic Features	Intestinal ^a	Gastric ^a	<i>p</i> -value
	N (%)	N (%)	
All Patients	57 (100)	85 (100)	
Age			
65	24 (42)	40 (48)	0.561
> 65	33 (58)	45 (53)	
Sex			
Female	10 (17)	14 (16)	0.867
Male	47 (83)	71 (84)	
Approach to Esophagectomy			
Open or Hybrid	14 (25)	12 (14)	0.115
Minimally Invasive	43 (75)	73 (86)	
History of Reflux Disease ^b			
Yes	49 (89)	70 (84)	0.428
No	6 (11)	13 (16)	
Barrett's esophagus			
Yes	54 (95)	75 (88)	0.188
No	3 (5)	10 (12)	
Body Mass Index ^c			
30	21 (42)	35 (44)	0.845
< 30	29 (58)	45 (56)	
Charlson Index ^d			
> 3	34 (61)	39 (46)	0.085
3	22 (39)	46 (54)	
Tumor Stage (T)			
1a (intramucosal)	20 (35)	19 (22)	0.096
1b (submucosal)	37 (65)	66 (78)	
Node Stage (N)			
N0	49 (86)	63 (74)	0.090
N1, N2 or N3	8 (14)	22 (26)	
Metastasis Stage (M)			
M0	57 (100)	84 (99)	1.000
M1	0 (0)	1 (1)	
Lymph Nodes Examined			
< 12	15 (26)	10 (12)	0.026
12	42 (74)	75 (88)	
Margin status			
Negative	56 (98)	84 (99)	1.000
Positive	1 (2)	1 (1)	

Clinical and Pathologic Features	Intestinal ^a	Gastric ^a	<i>p</i> -value
	N (%)	N (%)	
Tumor Location			
GE junction	37 (65)	51 (60)	0.554
Esophagus	20 (35)	34 (40)	
Tumor Size			
2 cm	31 (54)	42 (49)	0.561
> 2 cm	26 (46)	43 (51)	
Angiolymphatic Invasion			
No	46 (81)	65 (77)	0.550
Yes	11 (19)	20 (23)	
Tumor Grade			
Well or moderate	42 (74)	55 (65)	0.260
Poor	15 (26)	30 (35)	
Stage Group			
I	49 (86)	63 (74)	0.208
II	6 (11)	13 (15)	
III or IV	2 (4)	9 (11)	

^a Intestinal: MUC2+; Gastric: MUC2- and positive for gastric MUCs (includes 2 MUC null cases)

^b Data available for 138 of 142 patients

^c Data available for 130 of 142 patients

^d Data available for 141 of 142 patients

Table 2

Relationship of Histologic Pattern, CDX2 Expression and EGFR Amplification to the Mucin Immunophenotype

	MUC Phenotype		<i>p</i> -value
	Intestinal	Gastric	
	N (%)	N (%)	
All Patients	57 (100)	85 (100)	N/A
Histologic Pattern			
Tubulopapillary	46 (80)	79 (93)	<0.001
>10% Mucinous	10 (18)	1 (1)	
>10% Signet ring	1 (2)	0	
>10% Diffuse	0 (0)	5 (6)	
High CDX2 Expression ^a	22 (39)	13 (16)	0.003
EGFR Amplification ^b			
EGFR/CEP7 2	7/49 (14)	10/77 (13)	1.000
EGFR/CEP7 15	1/49 (2)	3/77 (4)	1.000

^a Average H score > 100 (i.e. > 50% of cells positive)

^b A total of 126 cases were successfully tested for EGFR amplification

Crude and Adjusted Cox Proportional Hazards Analysis of Overall Survival in Submucosally Invasive T1b EAC

Table 3

	Crude (Univariate)			Adjusted (Multivariate)		
	Hazard Ratio	95% CI	p	Hazard Ratio	95% CI	p
Intestinal phenotype ^a	2.0	1.1–3.6	0.028	2.2	1.2–4.2	0.010
Mass location (Esophagus) ^a	1.0	0.5–1.9	0.923			
Tumor size (cm) ^b	1.2	1.1–1.4	0.003	1.2	1.0–1.4	0.019
Poor differentiation ^a	1.4	0.8–2.6	0.264			
Angiolymphatic invasion ^{a,c}	3.1	1.7–5.8	<.001			
Lymph node metastasis ^a	2.9	1.6–5.4	0.001	2.5	1.3–4.8	0.003
Age (years) ^b	1.0	1.0–1.1	0.147			
Male sex ^a	1.2	0.6–2.7	0.613			
Charlson comorbidity index ^b	1.1	1.0–1.2	0.068			
Minimally invasive esophagectomy ^a	0.7	0.4–1.4	0.308			
12 LN resected ^a	0.8	0.3–1.7	0.496			

^a Variables entered as categorical: MUC2+ intestinal phenotype (vs MUC2- gastric); submucosal invasion (vs. intramucosal, T1a); mass location (esophagus vs. GE junction); poor differentiation (vs well or moderate differentiation); angiolymphatic invasion (vs no angiolymphatic invasion); lymph node metastasis (vs no lymph node metastasis); male sex (vs female); minimally invasive esophagectomy (vs other esophagectomy approach); 12 lymph nodes (vs < 12 lymph nodes).

^b Variables entered as continuous

^c Angiolymphatic invasion was not included in the multivariate analysis due to co-linearity with lymph node metastasis.