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## Tissue and serum mesothelin are potential markers of neoplastic progression in Barrett's-associated esophageal adenocarcinoma

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### Abstract

**Background**—Mesothelin is overexpressed in several malignancies and is purportedly a specific marker of malignant transformation. In this pilot study, we investigated whether tissue and serum mesothelin are potential markers of neoplastic progression in Barrett's esophagus (BE) and in esophageal adenocarcinoma (EAC).

**Methods**—Mesothelin expression was retrospectively evaluated in normal, BE, and EAC tissue from surgically resected esophageal specimens (n = 125). In addition, soluble mesothelin-related peptide (SMRP) levels were measured in serum.

**Results**—Normal esophageal mucosa did not express mesothelin. BE tissue with high-grade dysplasia specifically expressed mesothelin, whereas BE tissue with low-grade or without dysplasia did not. Fifty-seven (46%) EAC tumors were positive for mesothelin. EAC tumors with BE expressed mesothelin more often than those without BE (58% vs 35%, P = 0.01). SMRP levels were elevated in 70% of EAC patients (mean, 0.89 nM; range, 0.03–3.77 nM), but not in patients with acid reflux and/or BE.

**Conclusions**—Mesothelin is commonly expressed in BE-associated esophageal adenocarcinoma. Based on this pilot study, a prospective study is under way to evaluate tissue and serum mesothelin are potential markers of neoplastic progression in BE and in EAC (NCT01393483).

**Impact**—Current surveillance methods in Barrett's esophagus are invasive and neither cost-effective nor sensitive. This pilot study suggests that serum mesothelin is a marker of neoplastic transformation in BE and may provide a noninvasive method to improve identification of malignant transformation.

## Keywords

Mesothelin; SMRP; Barrett's esophagus; esophageal cancer; screening

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## INTRODUCTION

Barrett's esophagus (BE) is the strongest risk factor for and precursor of esophageal adenocarcinoma (EAC), which is associated with a poor overall prognosis (1, 2). Early detection of the metaplasia–dysplasia–adenocarcinoma sequence may help improve survival in patients with Barrett's–associated EAC (BEAC). However, endoscopic screening with biopsy, which is the standard surveillance technique for EAC, is limited by the low yield of quality tissue for detection of premalignant (dysplastic) mucosa because tissue samples are collected randomly and because dysplastic changes often occur in a spatially heterogeneous fashion (3, 4). This frequently results in a delay in the diagnosis of EAC (5). Furthermore, the histologic evaluation of excised esophageal mucosa for the presence of dysplasia is performed in a qualitative, subjective manner, and even among experienced pathologists, there is substantial intra- and interobserver variability in grading dysplasia (6). Thus, there is a clear need for a reliable screening modality that is capable of detecting malignant transformation in BE, which, in turn, may lead to the early diagnosis of BEAC.

Mesothelin, a cell-surface glycoprotein, has been shown to be overexpressed in several malignancies including mesothelioma, as well as pancreatic, ovarian, lung, and esophageal adenocarcinomas (7-9). Tissue mesothelin expression has been shown to be a specific marker of malignant transformation in colon and pancreatic cancer (7, 10, 11). In addition, serum mesothelin, which is detected as level of soluble mesothelin-related peptide (SMRP), has been shown to be a reliable tumor biomarker in several cancers (12, 13). In this pilot study, we sought to confirm that tissue mesothelin expression in esophageal tissue is specific for neoplastic progression to EAC. We also sought to demonstrate that serum mesothelin levels are elevated in EAC patients, but not in patients with acid reflux and/or BE without evidence of EAC.

## MATERIALS AND METHODS

### Study population

We retrospectively evaluated patients who underwent surgical resection for EAC or gastroesophageal junction adenocarcinoma at Memorial Sloan-Kettering Cancer Center between January 2005 and December 2007. In addition, we prospectively evaluated 10 patients undergoing surveillance endoscopy for acid reflux and/or BE without evidence of dysplasia or carcinoma. The institutional review board approved this study (IRB #09-169) and all patients provided informed written consent.

### Mesothelin immunohistochemistry

A total of 125 surgically resected esophageal specimens were available for the evaluation of mesothelin expression. Immunohistochemical analysis was performed on 4- $\mu$ m-thick sections of formalin-fixed, paraffin-embedded tissue using the avidin-biotin-peroxidase complex method. The primary antibody used was the anti-mesothelin monoclonal antibody (Vector Laboratories, Ltd, UK). For immunohistochemical staining, the sections were deparaffinized, rehydrated, and heated in sodium citrate buffer (pH 6.0) for antigen retrieval. The slides were then incubated in the primary antibody (1:90 dilution) for 60 minutes. Pancreatic adenocarcinoma tissue was used as the positive control.

A minimum of one slide with >80% tumor was evaluated for each specimen. For all specimens, areas of normal esophagus, BE, dysplasia, and EAC were evaluated for mesothelin expression. Mesothelin immunoreactivity was determined based on the percentage of reactive cells; staining was considered to be moderate-to-strong if >25% of the cells had cytoplasmic staining.

### Serum SMRP assay

A total of 131 EAC patients had serum available for SMRP determination. In addition, serum was obtained from the 10 patients with gastroesophageal reflux disease and/or BE without evidence of dysplasia or carcinoma. All blood samples were collected in EDTA tubes, centrifuged to separate serum, and stored at  $-20^{\circ}\text{C}$  until assayed.

SMRP levels were quantitatively measured using a double determinant (sandwich) immunoassay (MESOMARK®, Fujirebio Diagnostics, Inc., Malvern, PA, USA), per the manufacturer's instructions. This immunoassay has a six-point calibration curve (0–32 nM). Plates were read spectrophotometrically at 450nm using a BioTek ELx808 ultra microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

### Statistical analysis

Characteristics of patients with tumors that expressed mesothelin and of those with tumors that did not express mesothelin were compared using the Fisher's exact test (for categorical variables) and the non-parametric Wilcoxon rank-sum test (for continuous variables). Time to recurrence (TTR) after resection was estimated for each patient group using the Kaplan-Meier method and was compared between the two groups using the log-rank test. P-values of  $0.05$  were taken *a priori* to represent statistical significance.

## RESULTS

Baseline characteristics of patients with tissue available for mesothelin staining were comparable (supplemental table). In all tissue samples ( $n = 125$ ), areas of normal esophageal mucosa did not express mesothelin, whereas areas of high-grade dysplasia specifically expressed mesothelin, with both mucosal and intracellular mesothelin expression noted in adjacent EAC. Fifty-seven (46%) EAC tumors were positive for mesothelin, with a mean ( $\pm$  SD) of 29% ( $\pm 34\%$ ) of the cells stained (range, 0%–100%). Of EAC patients, patients with BE were more likely to express tissue mesothelin than those without BE (58% vs 35%,  $P = 0.01$ ). Moreover, in all patients with BEAC ( $n = 57$ ), areas of high-grade dysplasia stained positive for mesothelin, whereas areas of BE without dysplasia or with low-grade dysplasia did not (Fig. 1). In this cohort of patients, mesothelin expression was higher in smokers than in former and never smokers (71% vs 46% and 34%, respectively;  $P = 0.044$ ).

Of 131 EAC patients with serum available for SMRP measurement, 92 (70%) had elevated SMRP levels (our institution has previously published the normal interquartile range of serum SMRP to be 0.35–0.38 nM (14); Fig. 2) and 48% of these patients had SMRP levels twice above the normal range (>95% percentile of healthy individuals). In the 10 patients with acid reflux and/or BE without histologic evidence of dysplasia or carcinoma, SMRP levels were within the normal range in all cases (Table)— suggesting that elevation in serum mesothelin is specific to high-grade dysplasia in BE or EAC. In patients with EAC who received neoadjuvant chemoradiotherapy ( $n = 61$ ), the mean ( $\pm$  SD) SMRP level was  $0.91 \pm 0.67$  nM (range, 0.03–3.77 nM). The mean SMRP level at resection in EAC patients with stage T1 disease was 0.70 nM compared to 1.28 nM in EAC patients with stage T2-3 disease.

## DISCUSSION

Early detection of malignant transformation in BE may substantially reduce the overall mortality associated with EAC. However, standard endoscopic screening of patients with gastroesophageal reflux disease has proven to be neither sensitive nor cost-effective, subjecting many patients to invasive procedures with questionable benefit (4, 15). The reason is that most cases of BE will not progress to EAC and that standard four-quadrant biopsies can miss areas of occult dysplasia. In an effort to improve the current standard for EAC surveillance, several investigators have studied novel molecular and genetic markers of invasiveness and carcinogenesis (2). Alvarez et al. (16) previously reported specific mesothelin expression in an EAC cell line and in TMA tumor specimens. In the current study, we confirmed the specific expression of mesothelin in EAC cell lines (data not shown). Furthermore, in surgically resected esophageal specimens, we observed mesothelin expression in EAC and high-grade dysplastic tissue with >25% cell staining. However, none of the sampled areas of normal esophageal tissue or BE tissue with low-grade or without dysplasia stained positive for mesothelin. Unlike TMAs, the differential mesothelin expression in the same slide harboring normal, high-grade dysplastic, and EAC tissue is convincing and reproduced in multiple patients in our study.

Unlike tissue biomarkers, serum tumor biomarkers have been shown to be effective in screening populations at risk for several cancers such as prostate cancer (17) and mesothelioma (18), and thus may be well suited for use in EAC surveillance. In this study, we found that 70% of EAC patients had elevated SMRP levels, with the highest levels identified in those patients in whom EAC progressed from BE. These results suggest that serum mesothelin may be used to identify BE patients with neoplastic progression.

Recent studies have shown that complete pathologic response following neoadjuvant chemoradiotherapy for EAC may occur in as many as 26% of patients and is associated with improved 5-year survival (19). Standard imaging modalities for assessing treatment response in EAC include computed tomography, positron emission tomography, and endoscopic ultrasound. However, although these imaging techniques have been shown to be of prognostic value, they lack the sensitivity for detecting microscopic residual disease, which means that they cannot be used to assess the need for surgical resection or at least continued endoscopic surveillance following neoadjuvant therapy (20-23). In addition, our group recently reported that post-treatment endoscopic biopsy is also an unreliable method for predicting treatment response (24). Although the lack of tissue and serum collected at corresponding time points did not allow us to directly analyze whether tissue and serum mesothelin are predictive of recurrence or poor treatment response in the current study, our findings suggest that tissue mesothelin may help identify patients at high risk for recurrence.

In conclusion, mesothelin was expressed in nearly 60% of Barrett's-associated EAC patients. This finding suggests that mesothelin expression is a marker of malignancy and neoplastic transformation in esophageal tissue. This pilot study is limited because of its retrospective nature and heterogeneous patient population; however, our data suggest that detection of elevated serum mesothelin may provide a noninvasive marker of high-grade dysplasia or EAC and may play a role in the early detection of malignant transformation and/or progression. A prospective clinical trial is currently under way to evaluate the prognostic utility of tissue and serum mesothelin as markers of neoplastic progression in EAC patients ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01393483) identifier:NCT01393483).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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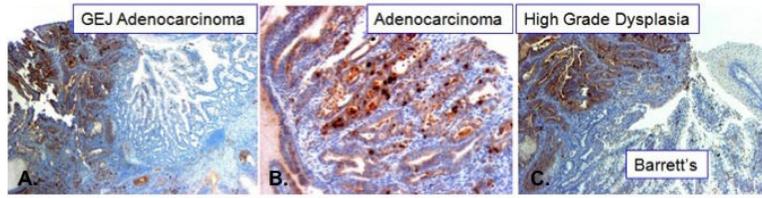
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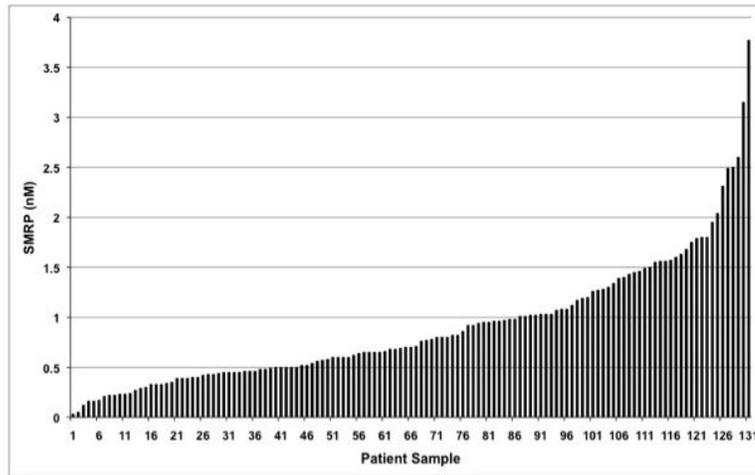
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**Figure 1.**

(A) Gastroesophageal junction and (B) distal esophageal adenocarcinoma tissue stained strongly for mesothelin. (C) Areas of high-grade dysplastic tissue also stained strongly for mesothelin, whereas areas of BE tissue did not.



**Figure 2.** SMRP levels measured in sera from EAC patients ( $n = 131$ ). In EAC patients with elevated SMRP levels ( $n = 92$ , 70%), mean SMRP level was 0.89 nM.

**Table**

SMRP levels in 10 patients without evidence of dysplasia or EAC who underwent endoscopic biopsy

Age (years)	Sex	Esophageal biopsy histology	Mean SMRP level (nM)
59	F	BE with focal low-grade dysplasia	0.5
46	F	BE, no dysplasia	0.4
64	F	BE, no dysplasia, esophagitis	0.5
73	F	BE, no dysplasia	0.6
42	M	BE, no dysplasia	0.5
74	M	BE, no dysplasia	0.3
38	M	Normal esophagus	0.3
59	F	Esophagitis	0.5
41	F	Mild chronic inflammation	0.6
56	M	Esophagitis	0.6