Published in final edited form as:

Am J Surg Pathol. 2015 April ; 39(4): 487–495. doi:10.1097/PAS.0000000000000356.

Smad4 Loss in Esophageal Adenocarcinoma Is Associated With an Increased Propensity for Disease Recurrence and Poor Survival

Aatur D. Singhi, MD, PhD^{*}, Tyler J. Foxwell, BS^{*}, Katie Nason, MD, MPH[†], Kristi L. Cressman, BA^{*}, Kevin M. McGrath, MD[‡], Weijing Sun, MD[‡], Nathan Bahary, MD, PhD[‡], Herbert J. Zeh, MD[†], Ryan M. Levy, MD[†], James D. Luketich, MD[†], and Jon M. Davison, MD^{*}

^{*}Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA

[†]Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA

[‡]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA

Abstract

Previously regarded as a rare neoplasm, the incidence of esophageal adenocarcinoma has risen rapidly in recent decades. It is often discovered late in the disease process and has a dismal prognosis. Current prognostic markers including clinical, radiographic, and histopathologic findings have limited utility and do not consider the biology of this deadly disease. Genome-wide analyses have identified *SMAD4* inactivation in a subset of tumors. Although Smad4 has been extensively studied in other gastrointestinal malignancies, its role in esophageal adenocarcinoma remains to be defined. Herein, we show, in a large cohort of esophageal adenocarcinomas, Smad4 loss by immunohistochemistry in 21 of 205 (10%) tumors and that Smad4 loss correlated with increased postoperative recurrence ($P=0.040$). Further, patients whose tumors lacked Smad4 had shorter time to recurrence (TTR) ($P=0.007$) and poor overall survival (OS) ($P=0.011$). The median TTR and OS of patients with Smad4-negative tumors was 13 and 16 months, respectively, as compared with 23 and 22 months, respectively, among patients with Smad4-positive tumors. In multivariate analyses, Smad4 loss was a prognostic factor for both TTR and OS, independent of histologic grade, lymphovascular invasion, perineural invasion, tumor stage, and lymph node status. Considering Smad4 loss correlated with postoperative locoregional and/or distant metastases, Smad4 was also assessed in a separate cohort of 5 locoregional recurrences and 43 metastatic esophageal adenocarcinomas. In contrast to primary tumors, a higher prevalence of Smad4 loss was observed in metastatic disease (44% vs. 10%). In summary, loss of Smad4 protein expression is an independent prognostic factor for TTR and OS that correlates with increased propensity for disease recurrence and poor survival in patients with esophageal adenocarcinoma after surgical resection.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

Correspondence: Aatur D. Singhi, MD, PhD, UPMC Presbyterian Hospital, 200 Lothrop Street, Room A616.2, Pittsburgh, PA 15213, singhiad@upmc.edu.

Conflicts of Interest: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Keywords

esophageal adenocarcinoma; Smad4; recurrence; locoregional; metastasis; survival

Esophageal cancer is the eighth most common cancer type and the sixth most common cause of cancer-related deaths worldwide.¹ Squamous cell carcinoma and adenocarcinoma account for >90% of all esophageal cancer cases. Internationally, squamous cell carcinoma is the most prevalent histologic subtype in the esophagus; however, in Western countries, esophageal cancer is a disease in epidemiologic transition. Whereas the incidence of squamous cell carcinomas has declined due to decreased smoking and alcohol consumption, the incidence of lower-third esophageal and gastroesophageal junction adenocarcinomas has risen rapidly.^{2,3} In the United States, the incidence of esophageal adenocarcinoma has increased by 600% in white male individuals over the last 30 years.⁴⁻⁶ The 5-year overall survival (OS) rate of esophageal adenocarcinoma is dismal at 17%, which is largely attributable to >50% of patients presenting with locally unresectable disease or distant metastasis.^{5,7,8} However, even in patients amenable to curative intervention with aggressive neoadjuvant therapy followed by surgery, the 5-year survival rates remain low at 30%.⁷ Current treatment algorithms rely on clinical, radiographic, and histopathologic assessments of disease to adequately prognosticate and stratify patients but have limited utility and do not consider the biology of this deadly disease.⁹

Advancements in whole-exome and whole-genome sequencing have uncovered the genetic landscape and spectrum of chromosomal alterations that characterize esophageal adenocarcinoma.¹⁰⁻¹² Recently, Dulak et al¹³ identified recurrent mutations within various members of the transforming growth factor beta (TGF- β)/Smad signaling pathway in 18% of esophageal adenocarcinomas. The most frequently altered gene in this pathway was *SMAD4*, which was mutated in 8% of tumors. In addition, *SMAD4* was subject to copy number loss in 34% of tumors. Smad4 is a key mediator of TGF- β signal transduction by heterodimerizing to receptor-regulated Smad proteins and translocating them to the nucleus.¹⁴ The Smad4/Smad complex then binds to specific DNA sequences and controls gene expression. Thus, many of the functions of TGF- β including growth suppression and cell death are abrogated with Smad4 loss.¹⁵

The relationship between *SMAD4* gene inactivation and tumorigenesis has been reported in other neoplasms, especially pancreatic ductal and colorectal adenocarcinomas.¹⁶⁻²¹ In both tumor types, somatic inactivation of *SMAD4* is accurately mirrored by loss of Smad4 protein expression by immunohistochemistry.^{22,23} Smad4 immunohistochemistry has proven to be a valuable tool in delineating the role of *SMAD4* in the pathogenesis of these tumors. In fact, the loss of Smad4 has been shown to correlate with progression to metastasis and poor prognosis. A similar association in esophageal adenocarcinomas has not been studied. We, therefore, evaluated Smad4 by immunohistochemistry in a large cohort of esophageal adenocarcinomas from patients who underwent potentially curative esophagectomy without induction therapy. Further, we assessed Smad4 in a separate cohort of locoregional recurrences and distant metastases and their corresponding primaries. These findings were

correlated with various clinicopathologic features including time to recurrence (TTR) and OS.

MATERIALS AND METHODS

Surgically Resected Esophageal Adenocarcinoma Study Cohort

Study approval was obtained from the University of Pittsburgh Institutional Review Board. Tissue microarrays were constructed from archival formalin-fixed paraffin-embedded (FFPE) tissue blocks from 159 esophageal adenocarcinoma resections seen at the University of Pittsburgh Medical Center between 1997 and 2009. Three, random 0.6-mm-sized cores were punched from each patient's tumor and harvested into recipient blocks.²⁴ Whole sections from an additional 46 esophageal adenocarcinoma resections were selected between the same time period. In total, 205 resected esophageal adenocarcinomas were obtained for immunohistochemical analysis. Representative hematoxylin and eosin-stained sections of each tumor were reviewed to confirm subtype, histologic grade, depth of invasion, lymph node status, and the presence of lymphovascular and perineural invasion. The pathologic primary tumor classification and stage groupings were determined according to the American Joint Committee on Cancer (AJCC) Staging Manual, seventh edition.²⁵ In addition, for each case, patient demographics, gross pathology reports, and prior pathology findings to include the identification of intestinal metaplasia were documented. Follow-up information was extracted from the patient's electronic medical record to include locoregional recurrence, distant metastasis, and survival. Further, patient survival information was cross-referenced with the US Social Security Death Index and medical records.

Locoregional and Distant Metastatic Esophageal Adenocarcinoma Study Cohort

A separate cohort of 5 locoregional recurrences and 43 distant metastases were identified within the surgical pathology archives at the University of Pittsburgh Medical Center among patients diagnosed between 1997 and 2013. All specimens in this cohort had pathologic confirmation of a primary esophageal adenocarcinoma by either biopsy or esophagectomy. Both hematoxylin and eosin slides and FFPE blocks were available for review and immunohistochemical analysis, respectively. Patient demographics, radiographic findings to document number of metastatic deposits, data regarding chronicity with respect to the patient's primary esophageal adenocarcinoma, and survival information were obtained from the medical record. In each case, an attempt was made to obtain FFPE blocks from the patient's corresponding primary esophageal adenocarcinoma. Matching primary tumors were also evaluated for all 5 locoregional recurrences and 28 of 43 (65%) distant metastases. None of these cases were within the initial surgically resected esophageal adenocarcinoma study cohort.

Immunohistochemical Analysis of Smad4 Expression

Immunohistochemical labeling was performed on 4- μ m-thick unstained sections. Slides were deparaffinized with serial xylene treatments and subjected to antigen retrieval using heated citrate solution (pH 9.0) at 100°C for 10 minutes. Immunolabeling for Smad4 (B-8 mouse monoclonal, dilution 1:500, Santa Cruz, CA) was performed on the automated

Ventana Benchmark XT system using the biotin-free Ventana OptiView DAB IHC Detection Kit (Ventana Medical Systems, Tucson, AZ). Immunohistochemical scoring of Smad4 expression was performed similar to those published previously.^{17,18} Assessment of Smad4 was done blinded to any other patient data including outcome. Intact or “positive” expression of Smad4 was defined as either nuclear or both nuclear and cytoplasmic staining within tumor cells, using stromal cells as a positive internal control (Figs. 1A, B). Loss or “negative” staining was scored in cases in which the tumor completely lacked both nuclear and cytoplasmic immunolabeling (Figs. 1C, D). In cases from constructed tissue microarrays, all 3 cores were required to demonstrate absence of staining within the tumor cells to be scored as negative. Similarly, negative staining in whole sections required loss of Smad4 expression within the entire tumor. Intratumoral heterogeneity was identified in 3 of 205 (1%) resected esophageal adenocarcinomas and scored as intact or “positive” expression for Smad4.

Statistical Analysis

χ^2 analysis or Fisher exact tests were used to compare categorical data, and analysis of variance was used to compare continuous variables. Survival curves were constructed using the Kaplan-Meier method, and differences between groups were evaluated by the log-rank test. TTR was calculated from the date of surgery to the date of first recurrence after surgery or to the date of last clinical evaluation for recurrence. OS was calculated as the time from the date of surgery to the date of death or the date of last follow-up. The prognostic significance of clinical and pathologic characteristics was determined using univariate Cox regression analysis. Multivariate analyses of significant risk factors were performed using Cox proportional hazard regression to identify independent risk factors for both TTR and survival. All statistical analyses were performed using the SPSS Statistical software, version 21 (IBM, Armonk, NY), and statistical significance was defined as a *P* value of <0.05.

RESULTS

Esophageal Adenocarcinoma Study Cohort

The clinical and pathologic features of the study cohort are summarized in Table 1. Patients at diagnosis ranged in age from 23 to 89 years (mean, 66.1 y) and were predominantly male (170 of 205, 83%). All 205 patients were treated by esophagectomy without neoadjuvant therapy. One hundred and fifty-two of 205 (74%) tumors were centered within the gastroesophageal junction, whereas the remaining 53 (26%) were located within the distal esophagus. Grossly, the tumors ranged in size from 0.5 to 13.0cm (mean, 4.6 cm). On the basis of the AJCC Staging Manual (seventh edition), 87 (42%) cases were histologically graded as well to moderately differentiated, and 118 (58%) were poorly differentiated. Lymphovascular and perineural invasion were identified in 158 (77%) and 94 (46%) tumors, respectively. Intestinal metaplasia (Barrett mucosa) was encountered in the background of 143 (70%) esophageal adenocarcinomas in either the surgical resection specimen or preoperative biopsies. The depth of tumor infiltration (pT staging) was classified as follows: 8 (4%) pT1a, 26 (13%) pT1b, 26 (13%) pT2, 141 (69%) pT3, and 4 (2%) pT4. Regional lymph node involvement by metastatic disease (pN staging) was identified in 179 (87%) patients and included 66 (32%) pN1, 57 (28%) pN2, and 56 (27%) pN3. Overall, 25 (12%)

patients had AJCC pathologic stage I disease, 19 (9%) stage II disease, and 161 (79%) stage III disease. No patients within the study cohort had stage IV disease. Postoperative locoregional recurrence and/or distant metastasis were identified in 102 (50%) patients. Among all 205 patients, the TTR rates were 62% at 1 year and 27% at 5 years, with a median of 18 months. The OS rates were 65% at 1 year and 21% at 5 years, with a median of 21 months.

Smad4 Immunohistochemistry and Correlation With Clinicopathologic Features

In total, 21 of 205 (10%) resected esophageal adenocarcinomas were categorized as negative by Smad4 immunohistochemical staining (Fig. 1). There were no statistically significant differences between Smad4 status and patient sex ($P=0.060$), age ($P=0.704$), tumor size ($P=0.755$), histologic grade ($P=0.065$), intestinal metaplasia in the background esophagus ($P=0.435$), presence of lymphovascular ($P=0.172$) and perineural ($P=0.165$) invasion, pT staging ($P=0.865$), pN staging ($P=0.174$), or AJCC pathologic stage ($P=0.843$).

However, Smad4 loss correlated with increased postoperative locoregional recurrence and/or distant metastases (71% vs. 47%, $P=0.040$). Patients whose tumors lacked Smad4 had shorter TTR and OS (Fig. 2). Among Smad4-negative esophageal adenocarcinoma patients, the TTR rates were 51% at 1 year and 6% at 5 years, with a median of 13 months. The OS rates were 57% at 1 year and 5% at 5 years, with a median of 16 months. In comparison, patients with Smad4-positive esophageal adenocarcinoma had significantly longer TTR (63% at 1 y and 29% at 5 y, median of 23 mo; $P=0.007$) and better OS (66% at 1 y and 23% at 5 y, median of 22 mo; $P=0.011$) rates.

Results from univariate Cox regression analysis for TTR and OS in relation to various clinicopathologic features including Smad4 immunohistochemical status are shown in Table 2. Shorter TTR and poor OS were associated with histologic grade ($P=0.001$ and $P<0.001$, respectively), lymphovascular invasion ($P<0.001$ and $P=0.001$, respectively), perineural invasion ($P<0.001$ and $P=0.001$, respectively), advanced T-stage (both $P<0.001$), advanced N-stage ($P=0.006$ and 0.003 , respectively), advanced overall AJCC pathologic stage ($P=0.019$ and 0.013 , respectively), and Smad4 loss by immunohistochemistry ($P=0.008$ and 0.012 , respectively). Multivariate analysis was also used to determine the prognostic significance of Smad4 loss for TTR and OS and included histologic grade, lymphovascular invasion, perineural invasion, tumor stage, lymph node metastasis, and Smad4 protein expression (Table 3). The loss of Smad4 expression was an independent prognostic factor for both TTR ($P=0.026$) and OS ($P=0.017$).

Assessment and Correlation of Smad4 Expression in Locoregional Recurrences and Metastases

Considering Smad4 loss in resected esophageal adenocarcinomas correlated with postoperative locoregional recurrence and/or distant metastases, the immunohistochemical status of Smad4 was assessed in separate cohorts of 5 locoregional recurrences and 43 distant metastases. Among the locoregional recurrences, all 5 patients had esophagogastrectomies with lymphadenectomy and negative surgical resection margins that ranged from 18 to 54 months (mean, 27.8 mo) before recurrence. The sites of locoregional

recurrence included 3 cervical lymph nodes and 2 esophagogastric anastomoses. Only 1 of 5 (20%) locoregional recurrences lacked Smad4 protein expression and was located at the esophagogastric anastomosis (Figs. 3A, B). The patient's corresponding primary surgical resection material also demonstrated loss of Smad4 (Figs. 3C, D), whereas the primaries from the remaining patients showed preserved Smad4 immunohistochemical staining.

The sites of distant metastases from 43 patients varied widely and included 21 (49%) liver, 7 (16%) lung, 6 (14%) peritoneum, 5 (12%) abdominal wall soft tissue, 2 (5%) omentum, 1 (2%) pleura, and 1 (2%) scalene muscle (Table 4). Although only 1 metastasis per patient was assessed for Smad4 expression, 25 (58%) patients had multiple metastatic deposits by radiographic imaging. In addition, 26 (60%) patients had synchronous primary and metastatic disease. Nineteen of 43 (44%) distant metastases were negative for Smad4 immunohistochemical staining (Figs. 4A, B). There were no statistically significant differences between Smad4 status in distant metastases and patient sex ($P=1.000$), mean age ($P=0.464$), number of metastatic deposits ($P=0.119$), chronologic presentation with respect to the patient's primary ($P=0.531$), or metastatic site ($P=0.501$). Corresponding primary esophageal adenocarcinoma biopsies or surgical resection material was available for Smad4 immunohistochemical analysis in 28 of 43 (65%) patients. Among these 28 primaries, 8 (29%) showed complete loss of Smad4 within the neoplastic cells (Figs. 4C, D), and all 8 corresponded to Smad4-negative metastases.

DISCUSSION

The National Cancer Institute defines a prognostic factor as “a situation or condition, or a characteristic of a patient, that can be used to estimate the chance of recovery from a disease or the chance of the disease recurring (coming back).” Current prognostic classification of esophageal adenocarcinoma relies on clinical measures (eg, patient age, functional and quality-of-life scores), radiologic characteristics (eg, endoscopic ultrasound locoregional staging), and pathologic findings (eg, TNM staging).²⁶ However, these parameters have limited ability in stratifying patients according to their likely outcome. Although patients with advanced-stage tumors clearly do worse than those with early disease, most patients with surgically resectable tumors present late, and, even within this group, there are wide variations in survival.⁹ Thus, additional markers of disease are required to accurately prognosticate patients with esophageal adenocarcinoma.

Herein, we identified loss of Smad4 protein expression in 10% of surgically resected esophageal adenocarcinomas. In addition, Smad4 loss correlated with shorter TTR and poor OS. The median TTR and OS of patients with Smad4-negative tumors were 13 and 16 months, respectively, as compared with 23 and 22 months, respectively, among patients with Smad4-positive tumors. In multivariate analysis, the absence of Smad4 expression was a negative, independent prognostic factor for both TTR and OS. These findings may be explained, at least in part, by the association of Smad4 loss with increased postoperative locoregional recurrence and distant metastases (71% vs. 47%).

Although recent genome-wide analyses of esophageal adenocarcinoma have identified recurrent genetic alterations in *SMAD4*, little has been reported with regard to Smad4 and its

role in the pathogenesis of esophageal adenocarcinoma.^{10,13} However, Smad4 has been extensively studied in other gastrointestinal malignancies. Originally isolated as a tumor-suppressor gene in pancreatic cancer, *SMAD4*, also termed *DPC4* (for *Deleted in Pancreatic Cancer, locus 4*), is inactivated in approximately 55% of pancreatic ductal adenocarcinomas, either by mutation with loss of heterozygosity or homozygous deletion.²⁷

Immunohistochemical staining for Smad4 directly mirrors its genetic status, and loss of protein expression serves as a surrogate marker for *SMAD4* inactivation.²³ Tascilar et al¹⁸ immunolabeled a large cohort of surgically resected pancreatic ductal adenocarcinomas for Smad4 and found that the absence of protein expression correlated with poor OS and served as a negative, independent prognostic factor. Analogous to esophageal adenocarcinoma, the association between Smad4 loss and a poor prognostic outcome may be related to an increased propensity for Smad4-negative pancreatic ductal adenocarcinomas to metastasize. Iacobuzio-Donahue et al²⁰ reported the patterns of metastasis in a series of patients with pancreatic cancer enrolled within a rapid autopsy program. The authors demonstrated that the absence of Smad4 expression by immunohistochemistry was often associated with death from widely metastatic disease, whereas patients with preserved Smad4 expression were more likely to have localized disease. Similar observations have also been made with colorectal adenocarcinoma. Both Maitra et al¹⁷ and Miyaki et al¹⁹ showed that the loss of Smad4 correlated with progression of colorectal cancer, especially distant metastasis. Both groups found no Smad4 inactivation in adenomas and stage I tumors, but the frequency of Smad4 loss increased with advanced disease. In addition, they found a higher frequency of Smad4 loss in colorectal adenocarcinomas with distant metastasis compared with those without distant metastasis. These findings parallel our own, wherein 44% of metastatic esophageal adenocarcinomas demonstrated SMAD4 loss. However, among the corresponding primaries, only 29% had SMAD4 inactivation. This suggests that loss of SMAD4 is a late event in the pathogenesis of esophageal adenocarcinoma.

The association of Smad4 loss with poor prognosis and distant metastasis has significant clinical implications. Surgical resection with curative intent remains the mainstay of definitive treatment for esophageal adenocarcinomas.²⁸ However, this can be a high-risk undertaking depending on surgeon experience and hospital volume. Although in-hospital mortality at our institution is <2%, it remains around 9% nationally.^{29,30} Moreover, significant morbidity as a consequence of postoperative complications can affect up to half of all patients who undergo surgery.³¹ Thus, a less aggressive course of treatment may be more appropriate for a subset of patients. Although further studies are required, the identification of a Smad4-negative esophageal adenocarcinoma in preoperative mucosal biopsies would indicate an increased risk for disease recurrence. In turn, this may prompt risk reassessment as to whether esophagectomy is beneficial to long-term oncologic outcomes. On the otherhand, a more aggressive approach may be indicated for Smad4-negative tumors.

The present study, however, is not without limitations. It is retrospective by design, and the number of patients is not large. Furthermore, not all patients within the study cohort received the same esophagectomy procedure (ie, open transhiatal or Ivor-Lewis esophagectomy vs. minimally invasive esophagectomy) or adjuvant therapy protocol. A

prospective validation of Smad4 protein expression as a prognostic biomarker in a large series of patients is clearly needed. In addition, according to the current National Comprehensive Cancer Network guidelines, preoperative neoadjuvant chemoradiation is preferred for most cases of esophageal adenocarcinoma.³² Patients receiving neoadjuvant treatment were specifically excluded from our study so as to not confound the interpretation of Smad4 loss. However, in other malignancies, the inactivation of Smad4 has been reported to contribute to chemoresistance and may show a similar correlation in esophageal adenocarcinoma.^{33,34}

In summary, we report the largest assessment of Smad4 protein status on cohorts of primary and recurrent esophageal adenocarcinomas. The loss of Smad4 protein expression is an independent prognostic factor for both shorter TTR and poor OS and correlates with increased propensity for disease recurrence in patients with esophageal adenocarcinoma after surgical resection. Although further studies are required, immunohistochemical analysis of Smad4 protein expression on preoperative biopsies may aid in stratifying patient treatment algorithms.

Acknowledgments

Source of Funding: Supported in part by a grant from the National Pancreas Foundation, Western Pennsylvania Chapter (to A.D.S.).

The authors would like to thank Mrs Robyn L. Roche for outstanding administrative assistance.

References

1. Ferlay, J.; Soerjomataram, I.; Ervik, M., et al. [Accessed March 8, 2014] Cancer incidence and mortality worldwide. IARC CancerBase No. 11. 2013. Available at: <http://globocan.iarc.fr>
2. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol*. 2006; 24:2137–2150. [PubMed: 16682732]
3. Vaughan TL, Davis S, Kristal A, et al. Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: adenocarcinoma versus squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*. 1995; 4:85–92. [PubMed: 7742727]
4. Blot WJ, Devesa SS, Kneller RW, et al. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *JAMA*. 1991; 265:1287–1289. [PubMed: 1995976]
5. Pohl H, Sirovich B, Welch HG. Esophageal adenocarcinoma incidence: are we reaching the peak? *Cancer Epidemiol Biomarkers Prev*. 2010; 19:1468–1470. [PubMed: 20501776]
6. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst*. 2005; 97:142–146. [PubMed: 15657344]
7. van Hagen P, Hulshof MC, van Lanschot JJ, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med*. 2012; 366:2074–2084. [PubMed: 22646630]
8. Enzinger PC, Mayer RJ. Esophageal cancer. *N Engl J Med*. 2003; 349:2241–2252. [PubMed: 14657432]
9. Rice TW, Rusch VW, Ishwaran H, et al. Worldwide Esophageal Cancer C. Cancer of the esophagus and esophagogastric junction: data-driven staging for the seventh edition of the American Joint Committee on Cancer/International Union Against Cancer Cancer Staging Manuals. *Cancer*. 2010; 116:3763–3773. [PubMed: 20564099]
10. Davison JM, Yee M, Krill-Burger JM, et al. The degree of segmental aneuploidy measured by total copy number abnormalities predicts survival and recurrence in superficial gastroesophageal adenocarcinoma. *PLoS One*. 2014; 9:e79079. [PubMed: 24454681]

11. Agrawal N, Jiao Y, Bettegowda C, et al. Comparative genomic analysis of esophageal adenocarcinoma and squamous cell carcinoma. *Cancer Discov.* 2012; 2:899–905. [PubMed: 22877736]
12. Dulak AM, Schumacher SE, van Lieshout J, et al. Gastrointestinal adenocarcinomas of the esophagus, stomach, and colon exhibit distinct patterns of genome instability and oncogenesis. *Cancer Res.* 2012; 72:4383–4393. [PubMed: 22751462]
13. Dulak AM, Stojanov P, Peng S, et al. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. *Nat Genet.* 2013; 45:478–486. [PubMed: 23525077]
14. Massague J. TGFbeta signalling in context. *Nat Rev Mol Cell Biol.* 2012; 13:616–630. [PubMed: 22992590]
15. Itoh S, Itoh F, Goumans MJ, et al. Signaling of transforming growth factor-beta family members through Smad proteins. *Eur J Biochem.* 2000; 267:6954–6967. [PubMed: 11106403]
16. Blackford A, Serrano OK, Wolfgang CL, et al. SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res.* 2009; 15:4674–4679. [PubMed: 19584151]
17. Maitra A, Molberg K, Albores-Saavedra J, et al. Loss of Dpc4 expression in colonic adenocarcinomas correlates with the presence of metastatic disease. *Am J Pathol.* 2000; 157:1105–1111. [PubMed: 11021814]
18. Tascilar M, Skinner HG, Rosty C, et al. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res.* 2001; 7:4115–4121. [PubMed: 11751510]
19. Miyaki M, Iijima T, Konishi M, et al. Higher frequency of Smad4 gene mutation in human colorectal cancer with distant metastasis. *Oncogene.* 1999; 18:3098–3103. [PubMed: 10340381]
20. Iacobuzio-Donahue CA, Fu B, Yachida S, et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol.* 2009; 27:1806–1813. [PubMed: 19273710]
21. Boone BA, Sabbhagan S, Zenati M, et al. Loss of SMAD4 staining in pre-operative cell blocks predicts distant metastases following pancreaticoduodenectomy with vascular resection for locally advanced pancreatic cancer. *J Surg Oncol.* 2014; 110:171–175. [PubMed: 24665063]
22. Montgomery E, Goggins M, Zhou S, et al. Nuclear localization of Dpc4 (Madh4, Smad4) in colorectal carcinomas and relation to mismatch repair/transforming growth factor-beta receptor defects. *Am J Pathol.* 2001; 158:537–542. [PubMed: 11159190]
23. Wilentz RE, Su GH, Dai JL, et al. Immunohistochemical labeling for dpc4 mirrors genetic status in pancreatic adenocarcinomas: a new marker of DPC4 inactivation. *Am J Pathol.* 2000; 156:37–43. [PubMed: 10623651]
24. Ong CA, Shapiro J, Nason KS, et al. Three-gene immunohistochemical panel adds to clinical staging algorithms to predict prognosis for patients with esophageal adenocarcinoma. *J Clin Oncol.* 2013; 31:1576–1582. [PubMed: 23509313]
25. Edge SB.; Byrd DR.; Compton CC., et al. *AJCC Cancer Staging Manual.* 7. New York, NY: Springer; 2010.
26. Weaver JM, Ross-Innes CS, Fitzgerald RC. The ‘-omics’ revolution and oesophageal adenocarcinoma. *Nat Rev Gastroenterol Hepatol.* 2014; 11:19–27. [PubMed: 23982683]
27. Hahn SA, Schutte M, Hoque AT, et al. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science.* 1996; 271:350–353. [PubMed: 8553070]
28. Wu PC, Posner MC. The role of surgery in the management of oesophageal cancer. *Lancet Oncol.* 2003; 4:481–488. [PubMed: 12901962]
29. Jamieson GG, Mathew G, Ludemann R, et al. Postoperative mortality following oesophagectomy and problems in reporting its rate. *Br J Surg.* 2004; 91:943–947. [PubMed: 15286953]
30. Luketich JD, Pennathur A, Awais O, et al. Outcomes after minimally invasive esophagectomy: review of over 1000 patients. *Ann Surg.* 2012; 256:95–103. [PubMed: 22668811]
31. Hulscher JB, van Sandick JW, de Boer AG, et al. Extended transthoracic resection compared with limited transhiatal resection for adenocarcinoma of the esophagus. *N Engl J Med.* 2002; 347:1662–1669. [PubMed: 12444180]
32. Cunningham D, Allum WH, Stenning SP, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med.* 2006; 355:11–20. [PubMed: 16822992]

33. Boulay JL, Mild G, Lowy A, et al. SMAD4 is a predictive marker for 5-fluorouracil-based chemotherapy in patients with colorectal cancer. *Br J Cancer*. 2002; 87:630–634. [PubMed: 12237773]
34. Zhang B, Chen X, Bae S, et al. Loss of Smad4 in colorectal cancer induces resistance to 5-fluorouracil through activating Akt pathway. *Br J Cancer*. 2014; 110:946–957. [PubMed: 24384683]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

FIGURE 1.

Smad4 immunohistochemistry in esophageal adenocarcinoma. Intact or “positive” immunolabeling for Smad4 was defined as nuclear or both nuclear and cytoplasmic staining within tumor cells and stromal cells, which served as a positive internal control (A, H&E; B, Smad4). Ten percent of resected esophageal adenocarcinomas were categorized as negative for Smad4. In these cases, complete absence of Smad4 immunolabeling within neoplastic cells and preserved staining in the surrounding stroma (C, H&E; D, Smad4) were observed. H&E indicates hematoxylin and eosin.

FIGURE 2.

Kaplan-Meier curves compare the cumulative probability of TTR (A) and OS (B) after esophagectomy among tumors with intact Smad4 (Smad4 positive) to those with loss of Smad4 (Smad4 negative). The P values were calculated using a log-rank test.

FIGURE 3.

A representative example of a mucosal biopsy demonstrating locoregional recurrence (A, H&E) at the site of anastomosis in a patient with a history of esophageal adenocarcinoma, status post esophagectomy with negative margins. Nuclear and cytoplasmic labeling for Smad4 is absent within the tumor cells, whereas intact staining was seen in the surrounding stroma and overlying squamous epithelium (B). Comparison of the patient's previously resected esophageal adenocarcinoma also demonstrates loss of Smad4 protein expression (C, H&E; D, Smad4). H&E indicates hematoxylin and eosin.

FIGURE 4.

An example of a patient presenting with esophageal adenocarcinoma and synchronous liver metastasis. A liver wedge biopsy of metastatic esophageal adenocarcinoma (A, H&E) that is negative for Smad4 by immunohistochemistry (B), whereas intact staining is seen within the uninvolved hepatocytes. Similarly, within a mucosal biopsy (C, H&E), Smad4 immunolabeling of the patient's primary esophageal adenocarcinoma shows loss of expression and preserved staining in the overlying squamous epithelium (D). H&E indicates hematoxylin and eosin.

TABLE 1

Clinical and Pathologic Comparison of Smad4-negative and Smad4-positive Esophageal Adenocarcinomas

Patient or Tumor Characteristics	Total, n = 205 (n [%])	Smad4 Negative, n = 21 (10%) (n [%])	Smad4 Positive, n = 184 (90%) (n [%])	P
Sex				
Female	35 (17)	7 (33)	28 (15)	0.060
Male	170 (83)	14 (67)	156 (85)	
Mean age (range) (y)	66.1 (23–89)	65.2 (44–85)	66.2 (23–89)	0.704
Mean tumor size (range) (cm)	4.6 (0.5–13.0)	4.5 (2.5–7.5)	4.6 (0.5–13.0)	0.755
Histologic grade				
Well to moderate	87 (42)	13 (62)	74 (40)	0.065
Poor	118 (58)	8 (38)	110 (60)	
Lymphovascular invasion				
Absent	47 (23)	2 (10)	45 (24)	0.172
Present	158 (77)	19 (90)	139 (76)	
Perineural invasion				
Absent	111 (54)	8 (38)	103 (56)	0.165
Present	94 (46)	13 (62)	81 (44)	
Primary tumor (pT) stage				
T1a	8 (4)	0 (0)	8 (4)	0.865
T1b	26 (13)	4 (19)	22 (12)	
T2	26 (13)	2 (10)	24 (13)	
T3	141 (69)	15 (71)	126 (69)	
T4	4 (2)	0 (0)	4 (2)	
Regional node (pN) stage				
N0	26 (13)	2 (10)	24 (13)	0.174
N1	66 (32)	3 (14)	63 (34)	
N2	57 (28)	7 (33)	50 (27)	
N3	56 (27)	9 (43)	47 (26)	
AJCC pathologic groupings				
Stage I	25 (12)	2 (10)	23 (13)	0.843
Stage II	19 (9)	1 (5)	18 (10)	
Stage III	161 (79)	18 (86)	143 (78)	
Postoperative disease recurrence				
Absent	103 (50)	6 (29)	97 (53)	0.040
Present	102 (50)	15 (71)	87 (47)	

P values in bold were statistically significant.

TABLE 2

Univariate Cox Regression Analysis of TTR and OS

Patient or Tumor Characteristics	TTR HR (95% CI)	<i>P</i>	OS HR (95% CI)	<i>P</i>
Sex, male vs. female	1.04 (0.64–1.70)	0.877	1.13 (0.75–1.69)	0.574
Age (y)	1.00 (0.98–1.13)	0.670	1.01 (0.99–1.03)	0.145
Histologic grade, poor vs. well or moderate differentiation	2.02 (1.35–3.03)	0.001	1.96 (1.42–2.71)	< 0.001
Lymphovascular invasion, presence vs. absence	2.54 (1.51–4.29)	< 0.001	1.94 (1.31–2.89)	0.001
Perineural invasion, presence vs. absence	2.30 (1.55–3.42)	< 0.001	1.67 (1.22–2.28)	0.001
Tumor stage (pT), pT3 and pT4 vs. pT1 and pT2	3.04 (1.86–4.97)	< 0.001	2.03 (1.40–2.94)	< 0.001
Lymph node metastasis (pN), pN3 and pN2 vs. pN1 and pN0	1.79 (1.18–2.72)	0.006	1.67 (1.19–2.34)	0.003
AJCC pathologic stage, stage III vs. stage I and II	3.49 (1.95–6.27)	< 0.001	2.25 (1.47–3.46)	< 0.001
Smad4 immunohistochemical staining, loss vs. preserved	2.11 (1.21–3.68)	0.008	1.80 (1.14–2.86)	0.012

P values in bold were statistically significant.

CI indicates confidence interval; HR, hazard ratio.

TABLE 3

Multivariate Cox Regression Analysis of TTR and OS

Patient or Tumor Characteristics	TTR HR (95% CI)	<i>P</i>	OS HR (95% CI)	<i>P</i>
Histologic grade, poor vs. well or moderate differentiation	1.64 (1.07–2.50)	0.024	1.76 (1.25–2.47)	0.001
Lymphovascular invasion, presence vs. absence	1.32 (0.72–2.41)	0.377	1.24 (0.77–1.98)	0.374
Perineural invasion, presence vs. absence	1.42 (0.91–2.24)	0.127	1.12 (0.78–1.60)	0.547
Tumor stage (pT), pT3 and pT4 vs. pT1 and pT2	1.81 (0.99–3.33)	0.055	1.42 (0.89–2.25)	0.143
Lymph node metastasis (pN), pN3 and pN2 vs. pN1 and pN0	1.26 (0.81–1.97)	0.304	1.27 (0.89–1.82)	0.186
Smad4 immunohistochemical staining, loss vs. preserved	1.95 (1.09–3.50)	0.026	1.79 (1.11–2.89)	0.017

P values in bold were statistically significant.

TABLE 4

Clinical and Pathologic Comparison of Metastatic Smad4-negative and Smad4-positive Esophageal Adenocarcinomas

Patient or Tumor Characteristics	Total, n = 43 (n [%])	Smad4 Negative, n = 19 (44%) (n [%])	Smad4 Positive, n = 24 (56%) (n [%])	P
Sex				
Female	3 (7)	1 (5)	2 (8)	1.000
Male	40 (93)	18 (95)	22 (92)	
Mean age (range) (y)	64.6 (47–83)	63.5 (50–75)	65.5 (47–83)	0.464
No. metastatic deposits				
Solitary	18 (42)	5 (26)	13 (54)	0.119
Multiple	25 (58)	14 (74)	11 (46)	
Presentation with respect to primary tumor				
Metachronous	17 (40)	9 (47)	8 (33)	0.531
Synchronous	26 (60)	10 (53)	16 (67)	
Primary tumor	28	14	14	
SMAD4 protein expression (n)				
Preserved	20 (71)	6 (43)	14 (100)	0.002
Loss	8 (29)	8 (57)	0 (0)	
Metastatic site				
Liver	21 (49)	7 (37)	14 (58)	0.501
Lung	7 (16)	5 (26)	2 (8)	
Peritoneum	6 (14)	3 (16)	3 (13)	
Abdominal wall soft tissue	5 (12)	3 (16)	2 (8)	
Omentum	2 (5)	1 (5)	1 (4)	
Pleura	1 (2)	0 (0)	1 (4)	
Scalene muscle	1 (2)	0 (0)	1 (4)	

P values in bold were statistically significant.