Published in final edited form as:

Pancreas. 2009 January ; 38(1): e1–e6. doi:10.1097/MPA.0b013e3181878b7f.

Epithelial-Mesenchymal Transition Markers in Pancreatic Ductal Adenocarcinoma

Justin M.M. Cates, MD, Ph^{*}, Robert H. Byrd, MD[†], Laurel E. Fohn, MD, PhD^{*}, Armanda D. Tatsas, MD^{*}, Mary K. Washington, MD, PhD^{*}, and Candice C. Black, DO[†]

^{*}Department of Pathology, Vanderbilt University Medical Center, Nashville, TN

[†]Department of Pathology, Dartmouth Hitchcock Medical Center, Lebanon, NH

Abstract

Objectives—Expression of transcription factors that mediate epithelial-mesenchymal transition (EMT), such as Twist and Slug, is correlated with poor prognosis in many tumor types. Selected EMT markers were studied in a series of pancreatic ductal adenocarcinomas (PDAs) and benign pancreatic tissues to determine whether expression levels correlated with diagnosis, histologic grade, or patient outcome.

Methods—Immunohistochemical stains for Twist, Slug, and N-cadherin were performed using a tissue microarray containing 68 PDAs and 38 samples of normal pancreas or chronic pancreatitis tissues.

Results—Twist and Slug were identified in both the nucleus and cytoplasm of benign pancreatic ductal epithelium, chronic pancreatitis, and PDA. Compared with normal ductal epithelium, nuclear levels of Twist are decreased in PDA. None of the other EMT markers showed significant differences in staining indices among the diagnostic groups. There were no correlations between EMT marker expression and histologic grade. Epithelial-mesenchymal transition marker expression was not associated with N-cadherin expression, patient outcome, or duration of survival.

Conclusions—Decreased expression of nuclear Twist is observed in malignant pancreatic epithelium. However, use of Twist as a diagnostic marker is precluded because decreased expression is also seen in chronic pancreatitis. None of the markers studied were predictive of patient outcome.

Keywords

epithelial-mesenchymal transition; pancreatic ductal adenocarcinoma

Despite advances in radiologic imaging procedures, the diagnosis of pancreatic ductal adenocarcinoma (PDA) remains difficult, particularly for tumors of low stage. Invasive diagnostic procedures such as endoscopic ultrasonography or computed tomography–guided fine-needle aspiration or core biopsy often yield ambiguous diagnoses such as “atypical” or “suspicious for malignancy” when the tumor is of low histologic grade (well-differentiated) or when the amount of diagnostic material is limited. Tumor markers that assist in the pathologic diagnosis of PDA would be clinically useful in this frequent and challenging clinical distinction.^{1–3}

Transcription factors that orchestrate epithelial-mesenchymal transition (EMT) have been correlated with increasing histologic grade and poor prognosis for several types of carcinoma. For example, increased expression of Snail, Slug, or Twist correlate with higher histologic grade and decreased relapse-free survival in patients with breast carcinoma.^{4–7} Expression of EMT markers is associated with higher tumor stage and shorter disease-free survival in esophageal, gastric, and colorectal carcinomas.^{8–11} Slug mRNA levels directly correlate with disease relapse and inversely correlate with survival in adenocarcinoma of the lung.¹² High levels of Twist have been reported as a poor prognostic factor in carcinomas of the female gynecologic tract.^{5,13–15} Twist is also up-regulated in prostatic adenocarcinoma of high Gleason grade and correlates with disease stage and histologic grade of urothelial carcinoma.^{16–18} Thus, much preliminary data suggest that these transcription factors may be useful as tumor markers.

Up-regulation of Twist mRNA in PDA compared with chronic pancreatitis, pancreatic intraepithelial neoplasia, and intraductal papillary mucinous neoplasms has been demonstrated by quantitative reverse transcription–polymerase chain reaction.¹⁹ However, these results were not corroborated when Twist expression in PDA was evaluated at the protein level by immunohistochemistry (IHC).²⁰ Snail and Slug expression have been documented in PDA, the former of which correlated with metastatic potential.^{20,21} These initial studies notwithstanding, the clinical and biologic significance of EMT marker expression in PDA is not fully understood. Therefore, expression of selected EMT transcription factors was evaluated by IHC in a series of 68 PDAs of varying histologic grade and compared with normal pancreatic epithelium and chronic pancreatitis. N-Cadherin (N-CDH), a putative phenotypic marker of EMT, was also evaluated.

MATERIALS AND METHODS

Case Retrieval

The surgical pathology files at the Vanderbilt University Medical Center were searched for pancreatic resection specimens performed for either PDA or nonneoplastic disease with existing tissue blocks suitable for construction of a tissue microarray (TMA). A total of 72 cases were identified, 4 of which were resected for benign disease (2 secondary to trauma, 1 for a benign pancreatic neoplasm, and 1 for a benign stricture of the common bile duct). Patient charts were reviewed to record pathologic data (histologic grade, tumor location, tumor size, and pTNM status), demographic data (patient age and sex), and follow-up information (outcome and interval from date of surgical resection to death). The study protocol was approved by the institutional review boards at both Vanderbilt University and Dartmouth College.

TMA Construction

All tissue samples were from surgical pathology specimens that had been fixed in neutral-buffered formalin and subsequently embedded in paraffin. The original hematoxylin and eosin–stained slides were reviewed by one of the authors (M.K.W.). Pathologic diagnoses were confirmed, PDAs were graded according to the World Health Organization criteria,²² and diagnostic areas were marked for TMA construction. A total of 64 cores of PDA, 25 cores of normal pancreatic ductal epithelium, and 13 cores of chronic pancreatitis were successfully harvested from the 68 pancreatic resection specimens. The TMA was constructed of 1.5-mm tissue cores using a manual arrayer (Beecher Instruments, Sun Prairie, Wis).

Immunohistochemical and Statistical Analyses

Tissue microarray slides were immunohistochemically stained for Twist (dilution at 1:200; Santa Cruz Biotechnology, Santa Cruz, Calif) or Slug (dilution at 1:100; Santa Cruz

Biotechnology) using previously described protocols.²³ Immunohistochemical staining for N-CDH (dilution at 1:100; Zymed, San Francisco, Calif) was performed after steam antigen retrieval in Trilogy buffer (Cell Marque, Rocklin, Calif) using the monoclonal EnVision + system and 3,3'-diaminobenzidine (Dako North America, Inc, Carpinteria, Calif) for antigen detection. Positive control sections for all antibodies were run in parallel. A TMA slide in which incubation with primary antibody was omitted served as the negative control. Snail was not evaluated in this series because satisfactory results could not be obtained on control stains with commercially available anti-Snail antibodies.

Tissue microarray slides were concurrently evaluated by 2 of the authors (R.H.B. and C.C.B.). Nuclear and cytoplasmic staining was scored using the Allred system with slight modifications.²⁴ Briefly, the staining index was considered as the sum of the intensity score (0, no staining; 1+, weak; 2+, moderate; 3+, strong) and the distribution score (0, no staining; 1+, staining of <33% of cells; 2+, between 33% and 66% of cells; and 3+, staining of >66% of cells). Staining indices were classified as follows: 4+ or higher, strong staining; 2+ to 3+, weak staining; and 0, negative staining. N-Cadherin was scored as positive if any detectable membranous staining was present. Epithelial-mesenchymal transition biomarker expression was compared with demographic and pathologic data by standard univariate nonparametric methods for ordinal data using XLStatistics software.²⁵ Bonferroni correction was applied for multiple independent comparisons. Post hoc analysis was performed using minimum significant differences in rank (minimum Q).²⁶

RESULTS

Clinicopathologic Data

The study group of PDAs represented on the TMA slides consisted of 68 cases (median patient age, 66 years; range, 37–84 years). Most tumors were poorly differentiated (grade 3, n = 30), 25 tumors were moderately differentiated (grade 2), and 13 tumors were well differentiated (grade 1). The pancreatic head was the most common site of origin (n = 49); 8 involved the pancreatic body and/or the tail and 11 were periampullary. Most of the cases were stage pT3, 4 were pT2, 1 was pT1, and 1 was pT4. Involvement of regional lymph nodes was detected in 38 patients at the time of resection. Two patients presented with distant metastatic disease within 6 weeks of surgery and were retrospectively considered stage IV at diagnosis. Another patient was upstaged to stage IV after ascites fluid was submitted at the time of surgery returned positive for adenocarcinoma. Therefore, most PDAs were AJCC TNM stage IIB (n = 36) or stage IIA (n = 25), 2 additional cases were stage III, and 1 case each was stages IA and IB.²⁷ The 7 non-stage II cases were excluded from the survival analysis to eliminate confounding effects. Four patients died of unrelated causes within a month of surgical resection and were excluded from the survival analyses. Median duration of follow-up for the remaining 57 cases was 19 months (range, 2–147 months), during which time 51 patients died.

Twist and Slug Expression in PDA

In our experience, intralesional heterogeneity of EMT marker expression is minimal.²³ Nonetheless, whole tissue sections of the interface between tumor and adjacent normal tissue representing the “invasive front” or “leading edge” of 5 different PDAs were stained with Twist, Slug, and N-CDH to assess potential regional variability in EMT marker staining. Similar IHC staining intensities and distributions were observed at the invasive fronts and within the central aspects of the tumors, confirming that there is minimal regional variability in staining indices for Twist or Slug within PDA (Fig. 1). Therefore, duplicate cores were not harvested during TMA construction.

Twist and Slug were detected by IHC in both pancreatic ductal and acinar epithelial cells from histologically normal pancreas and chronic pancreatitis and in PDAs of different histologic grades (Fig. 2). In most pancreatic tissue samples, ductal cells were positive for both nuclear and cytoplasmic Twist and Slug expression (Fig. 3). Kruskal-Wallis test demonstrated that only nuclear Twist levels varied significantly among the different tissues examined ($H = 19.86$; $P = 0.005$). Subsequent post hoc minimum significant differences analysis confirmed that nuclear Twist was decreased in PDA of all histologic grades compared with normal pancreatic ductal epithelium ($P < 0.01$) but not pancreatic ducts involved by chronic pancreatitis. There were no significant differences in nuclear Twist staining between PDAs of different histologic grade or between normal ductal epithelium and chronic pancreatitis.

The staining indices of cytoplasmic Twist, nuclear Slug, and cytoplasmic Slug did not vary significantly between the different diagnostic groups (Fig. 3). N-Cadherin was present in only 17% of PDAs and in 8% of chronic pancreatitis samples; none of the normal pancreatic tissues examined stained for N-CDH (Fisher exact test, $P = 0.04$). There were no statistically significant differences in N-CDH staining among PDAs of different histologic grade. None of the EMT markers studied showed statistically significant associations with N-CDH expression, patient age, patient sex, anatomic subsite (head, body/tail, perianipillary), tumor size, or AJCC stage.

In 24 cases, PDA (3 grade 1 tumors, 11 grade 2 tumors, and 10 grade 3 tumors) and adjacent benign ductal epithelium from the same pancreatic resection specimen were represented on the TMA for direct comparison. Net differences in the staining indices between neoplastic and adjacent benign epithelium were analyzed instead of ratios to avoid loss of cases because of division by 0. Staining index differences for nuclear Twist and nuclear Slug were significantly less than 0, indicating decreased expression in the malignant cells (Wilcoxon signed rank test, $P = 6.4 \times 10^{-5}$ and $P = 1.1 \times 10^{-4}$, respectively; Fig. 4). None of the staining index differences correlated with patient age, patient sex, anatomic sub-site, histologic grade, lymph node or distant metastasis, or N-CDH expression.

Twist and Slug as Prognostic Markers

None of the markers studied had a significant impact on patient outcome or duration of survival by Kaplan-Meier method. Additional analyses performed on the study cohort sub-stratified by histologic grade or AJCC stage confirmed that there were no statistically significant correlations between EMT marker expression and patient outcome or duration of survival for any of the subgroups analyzed. None of the EMT markers correlated with lymph node involvement or distant metastasis at presentation. In addition, the EMT marker staining index differences (in cases where PDA could be directly compared with adjacent benign ducts) did not correlate with either patient outcome or interval from surgery to death.

DISCUSSION

Strictly defined, EMT is a process by which epithelial cells lose contact with adjacent cells and basement membranes, switch patterns of extracellular matrix protein and intermediate filament expression, and transdifferentiate into mesenchymal cells. Although an essential and critical process of early embryonic development (ie, formation of mesoderm from epi-blast during gastrulation), whether true EMT occurs postnatally is controversial.^{28–32} Some investigators postulate that EMT represents a late event in the evolution of carcinoma and is responsible for the acquisition of an invasive, motile phenotype capable of distant metastasis.^{31,33–35} However, detection of EMT-related transcription factors in benign epithelium suggests that expression of these proteins alone is not sufficient for the acquisition of a spindle cell phenotype.^{6,18,19,36,37} Nevertheless, an increasing amount of evidence suggests that the transcription factors that coordinate EMT in utero (such as Twist, Slug, and Snail) are critical

in the progression of human malignancies and portend a worse prognosis. In this study, EMT markers were investigated in PDA and benign pancreas by IHC to determine whether these recently described markers are differentially expressed in PDA and whether their expression correlates with the degree of differentiation or prognosis.

EMT Markers in PDA and Benign Pancreas

Twist and Slug were identified in both the nuclei and cytoplasm of pancreatic ductal epithelial cells from histologically normal pancreas, chronic pancreatitis, and PDAs of all histologic grades. Although nuclear Twist levels were lower in PDA compared with normal pancreatic ductal epithelium, they were not significantly different from levels seen in chronic pancreatitis. Consequently, nuclear Twist expression is not useful in the differential diagnosis between low-grade PDA and reactive, nonneoplastic pancreatic ductal epithelium.

Most previous studies show that expression of EMT markers is increased in malignancy. In contradiction, our results demonstrate that expression levels of EMT markers in PDA are either similar to or are decreased compared with benign pancreatic ducts. The reason for this discrepancy is that, unlike pancreatic ductal epithelium, most other benign tissues studied to date (such as gastrointestinal, urothelial, and prostatic epithelium) do not express EMT transcription factors.^{8–11,16–18} Therefore, any alteration in EMT marker expression associated with neoplastic transformation of these tissues would inevitably manifest as increased expression by default. Previous studies of EMT marker expression in the pancreas corroborate our results. Quantitative real-time reverse transcription–polymerase chain reaction experiments have shown that levels of *Twist* mRNA are comparable in PDA and in nonneoplastic pancreas.¹⁹ Other investigators have reported low levels of Twist protein in PDA by IHC; comparison to normal ducts was not performed.²⁰ In addition to the pancreas, it has been reported that the intensity of Twist immunoreactivity in breast tumors is decreased compared with adjacent benign breast tissue.⁶

Correlation of EMT Markers With Histologic Grade

In certain types of carcinoma, increased expression of EMT markers has been correlated with higher histologic grade.^{4,6,10,16–18} Our results suggest that Twist and Slug expression does not vary among PDAs of different histologic grade. Similarly, a prior study showed that Snail expression in PDA does not correlate with tumor grade.²¹ Thus, it seems that Twist and Snail do not have a significant role in the degree of differentiation of PDA.

Correlation of EMT Markers With Prognosis

Increased levels of EMT markers have been correlated with poor prognosis in other tumor types.^{6,7,12,13} Expression of EMT markers in PDA did not correlate with patient outcome, duration of survival, or presence of metastatic disease at presentation. A prior study of EMT marker expression in PDA also failed to disclose significant correlations between Slug and tumor stage or lymph node metastasis.²⁰ In contrast, Snail expression has been correlated with lymph node involvement and distant metastasis in PDA by other investigators.²¹ Based on the available data, the clinical significance of altered levels of EMT transcription factors in PDA remains unclear.

Correlation of EMT Markers With N-CDH Expression

There is much evidence to suggest that Twist and Slug cause the morphologic changes of EMT by repressing E-cadherin and inducing expression of N-CDH, which decreases cell adhesion and increases cell motility.^{16,38–43} In PDA, however, N-CDH was detected in only a small minority of cases, and none of the EMT markers studied correlated with N-CDH expression. These results may be limited somewhat by the sensitivity of the polymer-based IHC detection

system used, because a prior study using a highly sensitive catalyzed tyramide signal amplification method demonstrated N-CDH expression in up to 43% of PDAs.⁴⁴ The absence of N-CDH expression in the TMA samples might also be an artifact of sampling bias. However, this possibly is not considered likely given the lack of regional variability in N-CDH staining in whole tissue sections of PDA (Fig. 1). Alternatively, expression EMT transcription factors alone may not be sufficient to initiate epithelial to mesenchymal transition in neoplastic pancreatic ductal epithelial cells.^{6,18,19,36,37}

Summary

Markers of EMT such as Twist and Slug are expressed in normal pancreatic ductal epithelium, chronic pancreatitis, and PDA. Levels of nuclear Twist showed significant variability among these different tissues. Although Twist is detectable in most pancreatic tissue samples, nuclear cytolocalization is significantly decreased in PDA compared with normal pancreatic ductal epithelium. However, similar patterns of staining for nuclear Twist in PDA and the reactive ducts seen in chronic pancreatitis preclude the diagnostic utility of this marker. Epithelial-mesenchymal transition marker expression did not correlate with patient outcome or overall survival. None of the EMT markers was associated with N-CDH expression. Further studies are warranted to examine the role of EMT in pancreatic development, repair, and carcinogenesis.

Acknowledgments

The authors thank Tony Frazier (Human Tissue Acquisition Laboratory and Pathology Core, Vanderbilt-Ingram Cancer Center) for construction of the TMA, Maudine Waterman (Research Pathology Services, Dartmouth-Hitchcock Medical Center) and Sandy Olson (Department of Pathology, Vanderbilt University Medical Center) for technical assistance, and Jean McClure (Department of Pathology, Vanderbilt University Medical Center) for editorial assistance.

This work was sponsored in part by GI SPORE P50 CA95103.

REFERENCES

1. Goggins M. Identifying molecular markers for the early detection of pancreatic neoplasia. *Semin Oncol* 2007;34:303–310. [PubMed: 17674958]
2. Goggins M. Molecular markers of early pancreatic cancer. *J Clin Oncol* 2005;23:4524–4531.
3. Yantiss RK, Woda BA, Fanger GR, et al. KOC (K homology domain containing protein overexpressed in cancer): a novel molecular marker that distinguishes between benign and malignant lesions of the pancreas. *Am J Surg Pathol* 2005;29:188–195. [PubMed: 15644775]
4. Blanco MJ, Moreno-Bueno G, Sarrio D, et al. Correlation of snail expression with histological grade and lymph node status in breast carcinomas. *Oncogene* 2002;21:3241–3246. [PubMed: 12082640]
5. Elloul S, Elstrand MB, Nesland JM, et al. Snail, slug, and smad-interacting protein 1 as novel parameters of disease aggressiveness in metastatic ovarian and breast carcinoma. *Cancer* 2005;103:1631–1643. [PubMed: 15742334]
6. Martin TA, Goyal A, Watkins G, et al. Expression of the transcription factors snail, slug, and twist and their clinical significance in human breast cancer. *Ann Surg Oncol* 2005;12:488–496. [PubMed: 15864483]
7. Moody SE, Perez D, Pan TC, et al. The transcriptional repressor snail promotes mammary tumor recurrence. *Cancer Cell* 2005;8:197–209.
8. Castro-Alves C, Rosivatz E, Schott C, et al. Slug is overexpressed in gastric carcinomas and may act synergistically with SIP1 and snail in the down-regulation of E-cadherin. *J Pathol* 2007;211:507–515.
9. Shioiri M, Shida T, Koda K, et al. Slug expression is an independent prognostic parameter for poor survival in colorectal carcinoma patients. *Br J Cancer* 2006;94:1816–1822. [PubMed: 16773075]

10. Uchikado Y, Natsugoe S, Okumura H, et al. Slug expression in the E-cadherin preserved tumors is related to prognosis in patients with esophageal squamous cell carcinoma. *Clin Cancer Res* 2005;11:1174–1180.
11. Yuen HF, Chan YP, Wong ML, et al. Upregulation of twist in oesophageal squamous cell carcinoma is associated with neoplastic transformation and distant metastasis. *J Clin Pathol* 2007;60:510–514. [PubMed: 16822877]
12. Shih JY, Tsai MF, Chang TH, et al. Transcription repressor slug promotes carcinoma invasion and predicts outcome of patients with lung adenocarcinoma. *Clin Cancer Res* 2005;11:8070–8078. [PubMed: 16299238]
13. Hosono S, Kajiyama H, Terauchi M, et al. Expression of twist increases the risk for recurrence and for poor survival in epithelial ovarian carcinoma patients. *Br J Cancer* 2007;96:314–320.
14. Kurrey NK, Amit K, Bapat SA. Snail and slug are major determinants of ovarian cancer invasiveness at the transcription level. *Gynecol Oncol* 2005;97:155–165. [PubMed: 15790452]
15. Kyo S, Sakaguchi J, Ohno S, et al. High twist expression is involved in infiltrative endometrial cancer and affects patient survival. *Hum Pathol* 2006;37:431–438. [PubMed: 16564917]
16. Kwok WK, Ling MT, Lee TW, et al. Up-regulation of TWIST in prostate cancer and its implication as a therapeutic target. *Cancer Res* 2005;65:5153–5162.
17. Yuen HF, Chua CW, Chan YP, et al. Significance of TWIST and E-cadherin expression in the metastatic progression of prostatic cancer. *Histopathology* 2007;50:648–658. [PubMed: 17394502]
18. Zhang Z, Xie D, Li X, et al. Significance of TWIST expression and its association with E-cadherin in bladder cancer. *Hum Pathol* 2007;38:598–606. [PubMed: 17258791]
19. Ohuchida K, Mizumoto K, Ohhashi S, et al. *Twist*, a novel oncogene, is upregulated in pancreatic cancer: clinical implication of twist expression in pancreatic juice. *Int J Cancer* 2007;120:1634–1640. [PubMed: 17236203]
20. Hotz B, Arndt M, Dullat S, et al. Epithelial to mesenchymal transition: expression of the regulators snail, slug, and twist in pancreatic cancer. *Clin Cancer Res* 2007;13:4769–4776. [PubMed: 17699854]
21. Yin T, Wang C, Liu T, et al. Expression of snail in pancreatic cancer promotes metastasis and chemoresistance. *J Surg Res* 2007;141:196–203. [PubMed: 17583745]
22. Klöppel, G.; Hruban, RH.; Longnecker, DS., et al. Ductal adenocarcinoma of the pancreas. In: Hamilton, SR.; Aaltonen, LA., editors. *World Health Organization Classification of Tumours. Pathology & Genetics of Tumors of the Digestive System*. Lyon, France: IARC Press; 2000. p. 221–230.
23. Cates JM, Dupont WD, Barnes JW, et al. Markers of epithelial-mesenchymal transition and epithelial differentiation in sarcomatoid carcinoma: utility in the differential diagnosis with sarcoma. *Appl Immunohistochem Mol Morphol* 2008;16:251–262. [PubMed: 18301244]
24. Allred DC, Clark GM, Elledge R, et al. Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *J Natl Cancer Inst* 1993;85:200–206. [PubMed: 8423624]
25. Version 06.08.27. Warrnambool, Australia: Deakin University; 2007. XLStatistics [computer program].
26. Dunn OJ. Multiple contrasts using rank sums. *Technometrics* 1964;6:241–252.
27. Greene, FL.; Page, DL.; Fleming, ID., et al. *AJCC Cancer Staging Manual*. 6th ed.. New York, NY: Springer-Verlag; 2002.
28. Barrallo-Gimeno A, Nieto MA. The snail genes as inducers of cell movement and survival: implications in development and cancer. *Development* 2005;132:3151–3161. [PubMed: 15983400]
29. Hemavathy K, Ashraf SI, Ip YT. Snail/slug family of repressors: slowly going into the fast lane of development and cancer. *Gene* 2000;257:1–12. [PubMed: 11054563]
30. Tarin D, Thompson EW, Newgreen DF. The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res* 2005;65:5996–6000. [PubMed: 16024596]
31. Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol* 2003;15:740–746. [PubMed: 14644200]

32. Thompson EW, Newgreen DF, Tarin D. Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? *Cancer Res* 2005;65:5991–5995. [PubMed: 16024595]
33. Lee JM, Dedhar S, Kalluri R, et al. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 2006;172:973–981. [PubMed: 16567498]
34. Guarino M, Rubino B, Ballabio G. The role of epithelial-mesenchymal transition in cancer pathology. *Pathology* 2007;39:305–318. [PubMed: 17558857]
35. Tse JC, Kalluri R. Mechanisms of metastasis: epithelial-to-mesenchymal transition and contribution of tumor microenvironment. *J Cell Biochem* 2007;101:816–829. [PubMed: 17243120]
36. Parent AE, Choi C, Caudy K, et al. The developmental transcription factor slug is widely expressed in tissues of adult mice. *J Histochem Cytochem* 2004;52:959–965.
37. Turner FE, Broad S, Khanim FL, et al. Slug regulates integrin expression and cell proliferation in human epidermal keratinocytes. *J Biol Chem* 2006;281:21321–21331.
38. Alexander NR, Tran NL, Rekapally H, et al. *N-Cadherin* gene expression in prostate carcinoma is modulated by integrin-dependent nuclear translocation of Twist1. *Cancer Res* 2006;66:3365–3369. [PubMed: 16585154]
39. Hajra KM, Chen DY, Fearon ER. The SLUG zinc-finger protein represses E-cadherin in breast cancer. *Cancer Res* 2002;62:1613–1618. [PubMed: 11912130]
40. Yang J, Mani SA, Donaher JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004;117:927–939.
41. Yang Z, Zhang X, Gang H, et al. Up-regulation of gastric cancer cell invasion by twist is accompanied by N-cadherin and fibronectin expression. *Biochem Biophys Res Commun* 2007;358:925–930.
42. Yokoyama K, Kamata N, Fujimoto R, et al. Increased invasion and matrix metalloproteinase-2 expression by snail-induced mesenchymal transition in squamous cell carcinomas. *Int J Oncol* 2003;22:891–898.
43. Rosivatz E, Becker I, Specht K, et al. Differential expression of the epithelial-mesenchymal transition regulators snail, *SIP1*, and twist in gastric cancer. *Am J Pathol* 2002;161:1881–1891. [PubMed: 12414534]
44. Nakajima S, Doi R, Toyoda E, et al. N-Cadherin expression and epithelial-mesenchymal transition in pancreatic carcinoma. *Clin Cancer Res* 2004;10:4125–4133. [PubMed: 15217949]

FIGURE 1.

Comparison of Twist, Slug, and N-CDH expression at the invasive front (interface between tumor and adjacent uninvolved pancreas) and the center of PDA. No differences in staining indices are demonstrable between tumor cells at the invasive front and those deeply embedded within the tumor. N-Cadherin highlights entrapped peripheral nerves and ganglion cells but does not stain tumor cells either at the invasive front or within the center of the tumor.

FIGURE 2.

Immunohistochemical staining for Twist and Slug in representative pancreatic tissues from the TMA, including benign ductal and acinar epithelium (Benign), chronic pancreatitis (CP), and PDA of increasing histologic grade (Gr 1 PDA to Gr 3 PDA). Nuclear staining for Twist in PDA is decreased in comparison to the nonneoplastic pancreatic ducts in the representative examples shown. Although Slug staining appears increased in PDA compared with benign pancreatic ductal epithelium, the observed differences in staining indices did not reach statistical significance.

FIGURE 3.

Prevalence of Twist, Slug, and N-CDH staining in human pancreatic tissues. The percentage of cases with negative staining (IHC staining index = 0), weak staining (2+ to 3+), or strong staining ($\geq 4+$) in each diagnostic group are displayed. N-Cadherin was scored as either positive or negative. For nuclear Twist, the distributions of staining indices among the diagnostic categories with letters in common are not statistically different by minimum significant differences in rank (minimum Q; $P < 0.01$). Nuclear Twist levels are lower in PDA than in benign pancreatic ducts but not those involved by chronic pancreatitis. Slug and cytoplasmic Twist levels were not significantly different among the different diagnostic groups. None of the EMT markers correlated with histologic grade.

FIGURE 4.

Box plots of the net differences in EMT marker staining index between PDA and adjacent benign pancreatic ductal epithelium retrieved from the same resection specimen ($n = 24$). The medians and interquartile ranges (box) are represented with the overall data range (lines); asterisks (*) denote outlier values, defined as more than 1.5 times the interquartile range from the lower or upper quartile. A bold line designates 0 or no net difference in staining index. Staining index differences for nuclear Twist and nuclear Slug were significantly less than 0, indicative of decreased expression in the malignant cells.