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Immunocytochemistry for MUC4 and MUC16 Is a Useful Adjunct in the Diagnosis of Pancreatic Adenocarcinoma on Fine-Needle Aspiration Cytology

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Abstract

Context.—Diagnoses rendered as atypical/suspicious for malignancy on fine-needle aspiration (FNA) of pancreatic mass lesions range from 2% to 29% in various studies. We have identified the expression of 3 genes, *MUC4*, *MUC16*, and *NGAL* that are highly upregulated in pancreatic adenocarcinoma. In this study, we analyzed the expression of these markers in FNA samples to determine whether they could improve sensitivity and specificity.

Objective.—To evaluate the utility of MUC4, MUC16, and NGAL in the evaluation of pancreatic FNA specimens.

Design.—Records of pancreatic FNAs performed during 10 consecutive years were reviewed. Unstained sections from corresponding cell blocks were immunostained for MUC4, MUC16, and NGAL (polyclonal). Immunostaining was assessed using the H-score (range, 0–3). Any case with an H-score of >0.5 was considered positive.

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Results.—Cases were classified using cytomorphologic criteria as adenocarcinoma (31 of 64; 48.4%), benign (17 of 64; 26.6%), and atypical/suspicious (16 of 64; 25%). On follow-up, all cases (100%; 31 of 31) diagnosed as carcinoma on cytology were confirmed on biopsy/resection samples or by clinical follow-up (such as unresectable disease). Of the cases diagnosed as atypical/suspicious, 69% (11 of 16) were found to be positive for adenocarcinoma and 31% (5 of 16) were benign on subsequent follow-up. Overall sensitivity and specificity, respectively, for the various markers for the detection of pancreatic adenocarcinoma were as follows: MUC4 (74% and 100%), MUC16 (62.9% and 100%), and NGAL (61.3% and 58.8%). In cases that were atypical/suspicious on cytology, expression of MUC4 and MUC16 was 100% specific for carcinoma with sensitivities of 63.6% and 66.7%, respectively.

Conclusion.—Immunocytochemistry for MUC4 and MUC16 appears to be a useful adjunct in the classification of pancreatic FNA samples, especially in cases that are equivocal (atypical/suspicious) for adenocarcinoma on cytomorphologic assessment.

Pancreatic adenocarcinoma is a leading cause of cancer-related death. According to estimates, approximately 43 140 new cases of pancreatic cancer were diagnosed in the United States in 2009, and an estimated 37 000 patients died from the malignancy in the same period.¹ Despite decades of research, the prognosis of patients diagnosed with pancreatic cancer remains bleak, with a median survival of only 6 months following diagnosis. Early tissue diagnosis of the disease can lead to improved chances for successful surgical/medical intervention. However, there is, as yet, no biomarker that has been shown to be sufficiently sensitive and specific to be applied to the general population. This, together with the relatively late clinical presentation of these patients, accounts for the poor prognosis of patients with pancreatic cancer.² Endoscopic ultrasound fine-needle aspiration (FNA) of pancreatic mass lesions is a safe, relatively noninvasive method for the diagnosis of pancreatic cancer. However, in about 2% to 29% of cases, the cytology is difficult to interpret, chiefly because of factors such as scant cellularity, blood cells obscuring cellular morphology, or morphologically equivocal cells in which a diagnosis of *atypical/suspicious cells for malignancy* is rendered.³⁻⁷ An equivocal diagnosis can preclude planning for definitive therapy. Molecular markers that are specific for pancreatic adenocarcinoma with a high sensitivity may greatly help in triaging cases that are equivocal on cytomorphologic assessment. To date, no immunocyto-chemical marker is being used routinely, to our knowledge, to confirm the diagnosis of pancreatic adenocarcinoma in FNA samples.

We have previously demonstrated that the expression of mucin-4 (MUC4), mucin-16 (MUC16), and neutrophil gelatinase-associated lipocalin (NGAL) is not expressed by the normal pancreatic ducts but is aberrantly expressed in the malignant ducts.⁸⁻¹³ MUC4 and MUC16 are transmembrane glycoproteins that, through alterations in the expression and nature of glycosylation, can affect cell adhesion, protein-protein interaction, and immune responses, all of which can facilitate tumor development and metastasis. MUC4 is normally expressed to some degree by the epithelium of the gastrointestinal tract, oral cavity, female reproductive tract, lacrimal glands, and middle ear; however, it is not expressed in the normal pancreatic ductal epithelial cells.⁹ Qualitative changes in expression of MUC4 have been described in various neoplastic processes.⁹ Aberrant MUC4 expression is detected in premalignant lesions preceding invasive pancreatic adenocarcinoma, termed *pancreatic intraepithelial neoplastic* (PanIN) lesions. It is increasingly expressed as the disease progresses¹⁴ and is, therefore, reported to be a marker of poor prognosis.¹⁵

MUC16 (commonly known as CA125) is expressed on the epithelial surfaces lining female reproductive organs, internal organs, and body cavities.⁹ An aberrant expression of MUC16 is reported in several epithelial malignancies, including ovarian cancer, where it is a well-known biomarker for following disease progression. MUC16 expression has also been

identified by immunocytochemistry in body-fluid aspirations from patients with metastatic pancreatic carcinoma.¹³

NGAL is a member of the lipocalin superfamily that functions primarily as an iron transporter, increasing cytoplasmic levels of the mineral. NGAL has been shown to be upregulated in various malignancies and has emerged as a poor prognostic indicator in patients with breast cancer.¹¹ In pancreatic carcinoma, we have shown that NGAL is strongly upregulated in the early stages of pancreatic dysplasia (ie, PanIN-1 or low-grade dysplasia) with increased expression in well-differentiated pancreatic cancer, followed by a loss of expression in more advanced/poorly differentiated adenocarcinoma.^{12,16}

Given the observation that these proteins are differentially expressed in pancreatic cancer tissues, we sought to investigate whether an immunocytochemistry panel using antibodies to these genes could serve as adjuncts in establishing a definitive diagnosis of malignancy on pancreatic FNA specimens, particularly those with a diagnosis of *atypical/suspicious for malignancy*.

MATERIALS AND METHODS

Approval for this study was obtained from our institutional review board. A computer search for all pancreatic fine-needle aspirations performed during a 10-year period at the Nebraska Medical Center was conducted. From this list of cases, only those that fulfilled the following criteria were selected for the study: (1) paraffin-embedded cell block was made from the aspiration material, and (2) cases diagnosed as atypical/suspicious had subsequent surgical/clinical diagnostic confirmation. Hematoxylin-eosin–stained slides from cell blocks from cases were, thus, selected for review to determine the presence or absence of diagnostic material. A total of 65 atypical/suspicious cases were reviewed, 16 (25%) of which met our criteria. Among these 16 cases, 11 (69%) were positive and 5 (31%) were negative for carcinoma on subsequent studies. Of 48 cases selected as positive and negative controls, which possessed adequate cell-block material, 31 (65%) were positive for adenocarcinoma, and 17 (35%) were negative for carcinoma on cytomorphologic assessment and confirmed by subsequent biopsy or clinical follow-up (at least 11 months without evidence of disease).

Unstained sections were immunostained for MUC4 (clone 8G7), MUC16 (clone M11, Dako, Carpinteria, California), and NGAL (polyclonal) using standard immunohistochemical methods. Anti-MUC4 was prepared in our laboratory. The anti-NGAL antibody was acquired from M. Gould, PhD.¹⁷ An H-score (range, 0–3) was used to assess the immunohistochemical staining. H-score is a summation of the product of staining intensity (range, 0–3) and proportion of cells staining (range, 0–1).¹⁸ Two pathologists scored all samples independently, and discrepancies were resolved by simultaneous examination. A receiver operator characteristic curve analysis comparing pancreatic FNA specimens with known adenocarcinoma and benign samples was conducted using GraphPad Prism 5.0 (GraphPad Software, Inc, La Jolla, California). Following this analysis, any case with an H-score of greater than 0.5 was considered a positive result. Because of technical issues, such as exhausted paraffin blocks or lack of abnormal cells in deeper cut sections, immunostaining for all of the markers was not possible on some specimens. The final number of cases available for evaluation for each marker is as follows: MUC4: adenocarcinoma, 30; negative for carcinoma, 15; atypical/suspicious, 16; MUC16: adenocarcinoma, 27; negative for carcinoma, 15; atypical/suspicious, 15; and NGAL: adenocarcinoma, 31; negative for carcinoma, 17; atypical/suspicious, 16.

RESULTS

Immunocytochemical staining for all 3 markers when present was cytoplasmic (Figures 1, A through C, 2, A through C, 3, A through C, and 4, A through C). When staining was present/positive, it was strong (of high intensity) and distinct from the surrounding debris and normal or benign epithelium. The surrounding normal gastrointestinal epithelium did not stain or stained with a very weak intensity, which was similar to that seen in surrounding debris/background. Any case with an H-score of greater than 0.5 was considered positive. The mean H-score for the 3 markers in all cases, positive or negative, respectively, for adenocarcinoma is as follows: MUC4 (1.76, 0.11), MUC16 (1.12, 0), and NGAL (0.94, 0.43). In atypical/suspicious cases, the mean H-score for those cases on follow-up found to be positive or negative for malignancy, respectively, was as follows: MUC4 (1.72, 0), MUC16 (1.13, 0), and NGAL (1.09, 0.44) (Figure 5). The sensitivity and specificity, respectively, of the 3 markers for the diagnosis of adenocarcinoma on cytology are as follows: MUC4 (74%, 100%), MUC16 (62.9%, 100%), and NGAL (61.3%, 58.8%). In atypical/suspicious cases, the sensitivity and specificity, respectively, of the markers were as follows: MUC4 (63.6%, 100%), MUC16 (66.7%, 100%), and NGAL (54.5%, 60%) (Table). There was no morphologic change that could be identified in the tumors that were negative for MUC4 or MUC16.

COMMENT

Treatment of pancreatic carcinoma requires a positive tissue diagnosis. Endoscopic ultrasound FNA of pancreatic mass lesions is a relatively noninvasive method for the diagnosis of pancreatic cancer. However, its sensitivity ranges from 78% to 94% in various studies with a reported false-negative rate of about 3% to 5%. Diagnoses rendered as suspicious/atypical range from 2% to 29%.^{3,4} Pancreatic FNAs can suffer from the shortcomings of inadequate sampling and indeterminate results on cytology. Although on-site assessment of the FNA specimens has been shown to be useful in improving the diagnostic performance of endoscopic ultrasound-guided FNAs, the procedure is highly dependent on the availability of adequately trained personnel and staffing. Many centers do not have cytopathologists available on site for adequate assessment of samples. An inadequate sample, with scant atypical cells, coexistence of gastrointestinal epithelium and inflammatory cells, poor cellular preservation, combined with erroneous interpretation, are some of the reasons for an indeterminate diagnosis on cytologic assessment. Although these limitations have been well recognized for many years, there are, currently, no molecular markers that are available for use on a routine basis in diagnostically difficult cases to improve the sensitivity and specificity of a morphologic diagnosis. In our study, we examined the immunocytochemistry staining characteristics of MUC4, MUC16, and NGAL in pancreatic FNA specimens. Our results suggest that MUC4 and MUC16 are 100% specific in the diagnosis of pancreatic cancer in “atypical/suspicious” cases and, thus, could potentially be useful in the diagnosis of pancreatic carcinoma in indeterminate cases.

MUC4 has previously been described as a potential tissue-based diagnostic marker for pancreatic cancer, and its expression increases progressively in PanIN lesions,¹⁴ with the highest expression noted in well-differentiated pancreatic adenocarcinoma.¹⁵ In an earlier study, MUC4 could distinguish reactive cells in FNA specimens of patients with chronic pancreatitis from those of adenocarcinoma, with a reported overall sensitivity and specificity of 91% and 100%, respectively.⁸ The sensitivity and specificity for adenocarcinoma within the atypical/suspicious group was 80% and 100% in that study. Our study confirms the high specificity for MUC4 in the diagnosis of malignancy; however, we observed a lower sensitivity overall (74%) and specifically within the atypical/suspicious category (63.6%). Nonetheless, the highly specific nature of staining for MUC4 can be exploited to triage and

reclassify cases with an atypical/suspicious cytomorphologic diagnosis, limiting the need for additional diagnostic procedures and health care costs.

MUC16 has been reported as a marker of pancreatic adenocarcinoma in effusion aspirations, with a reported sensitivity of approximately 90%.¹³ To our knowledge, no study has evaluated MUC16 immunocytochemistry as an adjunct in the cytologic assessment of pancreatic FNA samples. The finding of 100% specificity for adenocarcinoma in both the “atypical/suspicious” and “positive for pancreatic adenocarcinoma” categories in our study supports the utility of MUC16 as an adjunctive test in confirming the diagnosis of adenocarcinoma in difficult cases.

NGAL expression had a low sensitivity and specificity in both cases diagnosed as positive on cytology (61.3% and 58.8%) and in cases with a diagnosis of atypical/suspicious (54.5% and 60%). One likely explanation for the lower sensitivity and specificity for NGAL expression in our study is that NGAL has been reported to be expressed in PanIN lesions and well-differentiated adenocarcinomas, with loss of expression as the tumor becomes more poorly differentiated.^{12,16} Further, NGAL is expressed by inflammatory cells, particularly polymorphonuclear leukocytes. This may contribute to the lower specificity of NGAL in pancreatic cancer FNA.

One limitation to the current study as well as the only prior study of MUC4 in FNA samples is the relatively few atypical/suspicious FNA specimens. Our strict exclusion criteria and the relative paucity of cells (on the smear or the cell block) which limits assessment by immunocytochemistry are the chief reasons for the few examined cases. However, both studies confirmed the high specificity of MUC4 in the diagnosis of pancreatic adenocarcinoma, whereas our study demonstrated the novel finding that MUC16, another membrane-bound mucin, is equally specific for malignant cells in FNA specimens. We suggest that intraprocedural assessment of FNA samples and the use of immunocytochemical staining with anti-MUC4 and anti-MUC16 antibodies may potentially increase the sensitivity of pancreatic cancer diagnosis. Further, additional studies optimizing the conditions for immunocytochemistry for the 2 markers on Papanicolaou-stained slides, which may contain greater amounts of lesional tissue, may further help to increase their clinical diagnostic utility.

To conclude, immunocytochemistry for MUC4 and MUC16 is a useful adjunct in the cytomorphologic assessment of pancreatic FNA samples. Their expression could be used to confirm cases positive by morphologic assessment and to further classify cases with an atypical/suspicious diagnosis. The latter scenario is especially important because it would obviate the need for a repeat biopsy and, thereby, greatly help contain health care costs.

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References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin.* 2010; 60(5):277–300. [PubMed: 20610543]
2. Chakraborty S, Baine MJ, Sasson AR, Batra SK. Current status of molecular markers for early detection of sporadic pancreatic cancer. *Biochim Biophys Acta.* 2011; 1815(1):44–64. [PubMed: 20888394]

3. Eloubeidi MA, Jhala D, Chhieng DC, et al. Yield of endoscopic ultrasound-guided fine-needle aspiration biopsy in patients with suspected pancreatic carcinoma. *Cancer*. 2003; 99(5):285–292. [PubMed: 14579295]
4. Gress F, Gottlieb K, Sherman S, Lehman G. Endoscopic ultrasonography-guided fine-needle aspiration biopsy of suspected pancreatic cancer. *Ann Intern Med*. 2001; 134(6):459–464. [PubMed: 11255521]
5. Eltoun IA, Chhieng DC, Jhala D, et al. Cumulative sum procedure in evaluation of EUS-guided FNA cytology: the learning curve and diagnostic performance beyond sensitivity and specificity. *Cytopathology*. 2007; 18(3):143–150. [PubMed: 17388936]
6. Fisher L, Segarajasingam DS, Stewart C, Deboer WB, Yusoff IF. Endoscopic ultrasound guided fine needle aspiration of solid pancreatic lesions: performance and outcomes. *J Gastroenterol Hepatol*. 2009; 24(1):90–96. [PubMed: 19196396]
7. Toucheffeu Y, Le Rhun M, Coron E, et al. Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis of solid pancreatic masses: the impact on patient-management strategy. *Aliment Pharmacol Ther*. 2009; 30(10):1070–1077. [PubMed: 19735232]
8. Jhala N, Jhala D, Vickers SM, et al. Biomarkers in diagnosis of pancreatic carcinoma in fine-needle aspirates. *Am J Clin Pathol*. 2006; 126(4):572–579. [PubMed: 17019794]
9. Bafna S, Kaur S, Batra SK. Membrane-bound mucins: the mechanistic basis for alterations in the growth and survival of cancer cells. *Oncogene*. 2010; 29(20):2893–2904. [PubMed: 20348949]
10. Moniaux N, Andrianifahanana M, Brand RE, Batra SK. Multiple roles of mucins in pancreatic cancer, a lethal and challenging malignancy. *Br J Cancer*. 2004; 91(9):1633–1638. [PubMed: 15494719]
11. Bolignano D, Donato V, Lacquaniti A, et al. Neutrophil gelatinase-associated lipocalin (NGAL) in human neoplasias: a new protein enters the scene. *Cancer Lett*. 2010; 288(1):10–16. [PubMed: 19540040]
12. Tong Z, Kunnumakkara AB, Wang H, et al. Neutrophil gelatinase-associated lipocalin: A novel suppressor of invasion and angiogenesis in pancreatic cancer. *Cancer Res*. 2008; 68(15):6100–6108. [PubMed: 18676832]
13. Han L, Pansare V, Al-Abbadi M, Husain M, Feng J. Combination of MUC5ac and WT-1 immunohistochemistry is useful in distinguishing pancreatic ductal carcinoma from ovarian serous carcinoma in effusion cytology. *Diagn Cytopathol*. 2010; 38(5):333–336. [PubMed: 19856421]
14. Swartz MJ, Batra SK, Varshney GC, et al. MUC4 expression increases progressively in pancreatic intraepithelial neoplasia. *Am J Clin Pathol*. 2002; 117(5):791–796. [PubMed: 12090430]
15. Westgaard A, Schjolberg AR, Cvancarova M, Eide TJ, Clausen OP, Gladhaug IP. Differentiation markers in pancreatic head adenocarcinomas: MUC1 and MUC4 expression indicates poor prognosis in pancreatobiliary differentiated tumours. *Histopathology*. 2009; 54(3):337–347. [PubMed: 19236510]
16. Moniaux N, Chakraborty S, Yalniz M, et al. Early diagnosis of pancreatic cancer: neutrophil gelatinase-associated lipocalin as a marker of pancreatic intraepithelial neoplasia. *Br J Cancer*. 2008; 98(9):1540–1547. [PubMed: 18392050]
17. Stoesz SP, Friedl A, Haag JD, Lindstrom MJ, Clark GM, Gould MN. Heterogeneous expression of the lipocalin NGAL in primary breast cancers. *Int J Cancer*. 1998; 79(6):565–572. [PubMed: 9842963]
18. Wilder JL, Shajahan S, Khattar NH, et al. Tamoxifen-associated malignant endometrial tumors: pathologic features and expression of hormone receptors estrogen- α , estrogen- β and progesterone; a case controlled study. *Gynecol Oncol*. 2004; 92(2):553–558. [PubMed: 14766247]

Figure 1.
A through C, Pancreatic ductal adenocarcinoma, diagnosed on cytology: A, hematoxylin-eosin; B, anti-MUC4; and C, anti-MUC16 (original magnifications $\times 400$).

Figure 2.
A through C, Benign pancreatic ductal epithelium: A, hematoxylin-eosin; B, anti-MUC4;
and C, anti-MUC16 (original magnifications $\times 400$).

Figure 3. A through C, Atypical pancreatic ductal epithelium, diagnosed as adenocarcinoma on surgical follow-up: A, hematoxylin-eosin; B, anti-MUC4; and C, anti-MUC16 (original magnifications $\times 400$).

Figure 4. A through C, Atypical pancreatic ductal epithelium, diagnosed as pancreatitis on surgical follow-up: A, hematoxylin-eosin; B, anti-MUC4; and C, anti-MUC16 (original magnifications $\times 200$).

Figure 5.
Mean H-scores for MUC4, MUC16, and NGAL from all cases.

Sensitivity and Specificity for Adenocarcinoma for MUC4, MUC16, and NGAL in All Cases and in Cases Diagnosed as Atypical/Suspicious

	MUC4, %	MUC16, %	NGAL, %
Sensitivity (all cases)	74	62.9	61.3
Specificity (all cases)	100	100	58.5
Sensitivity (atypical cases)	63.6	66.7	54.5
Specificity (atypical cases)	100	100	60