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Review Article

Morphologic Variants of Well-Differentiated Pancreatic Neuroendocrine Tumors: Fine-Needle Aspiration Cytomorphology and Diagnostic Pitfalls

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Abstract

Morphologic variants of well-differentiated pancreatic neuroendocrine tumors (PanNETs) are uncommon. These variants may mimic non-PanNETs, and when under recognized, it would lead to misdiagnosis by fine-needle aspiration (FNA) biopsy. The present report describes the unique cytomorphologic features and diagnostic clues of pigmented, pleomorphic, clear cell/lipid-rich and oncocytic variants of well-differentiated PanNETs. The differential diagnoses of each morphologic variant are also discussed. Ancillary immunohistochemical studies with appropriate markers are crucial in the diagnostic work-up. Raising the awareness of PanNET morphologic variants is essential for preventing diagnostic pitfalls, and rendering an accurate diagnosis during the FNA diagnostic work-up of pancreatic lesions.

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Introduction

The vast majority of pancreatic neuroendocrine tumors (PanNETs) are well-differentiated, and are sub-classified as functional or non-functional tumors, together accounting for approximately 2% of all pancreatic tumors.[1] The preferred preoperative approach for the diagnosis of PanNET is endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA).[2–6] Well-differentiated PanNETs have unique cytomorphologic features. The classic features include single and dyscohesive clusters of relatively uniform tumor cells with eosinophilic cytoplasm, eccentrically located nuclei (plasmacytoid appearance), and speckled (salt and pepper) chromatin. With the recognition of these cytomorphologic features, the application of confirmatory immunohistochemistry studies with neuroendocrine markers has resulted in accurate cytological diagnoses.

However, a spectrum of morphologic variants of PanNETs, although very uncommon, has been gradually recognized, and some of these variants have distinct biologic and prognostic significance.[7–14] More importantly, these variants may not retain the classic cytomorphologic features of PanNETs. Therefore, these may present as substantial diagnostic challenges, especially in fine-needle aspiration (FNA) specimens, when under recognized. Raising the awareness of the characteristic morphology of individual variants is the most effective approach to prevent a potential misdiagnosis. The present review discussed examples of selective PanNET morphologic variants, with focus on the specific cytomorphology and corresponding differential diagnosis.

Pigmented variant

Although the pigmented variant may largely retain the classic cytomorphology of PanNETs, the cells of this variant additionally and characteristically contain abundant brown-to-black granular pigments in the cytoplasm, often obscuring nuclear details (Fig. 1). The pigment is usually also present in the background, which is presumably caused by the dispersal of the pigment from ruptured cells during smearing.[15,16]

The presence of brown-black pigment can easily raise the differential diagnosis of malignant melanoma, which is the most common pigmented neoplasm secondarily involving the pancreas. Malignant melanoma demonstrates overlapping cytomorphologic features with PanNETs, which include highly cellular aspirates, isolated-to-loosely cohesive cells, eccentric nuclei presenting with a plasmacytoid appearance, and occasional binucleated cells. The pigments in these two entities are morphologically indistinguishable. However, as observed on electron microscopy, the pigment in PanNETs is neuromelanin or lipofuscin, and not melanin seen in malignant melanoma.[9,15] An easily accessible immunohistochem-

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Abbreviations: FNA, fine-needle aspiration; PanNET, pancreatic neuroendocrine tumor.
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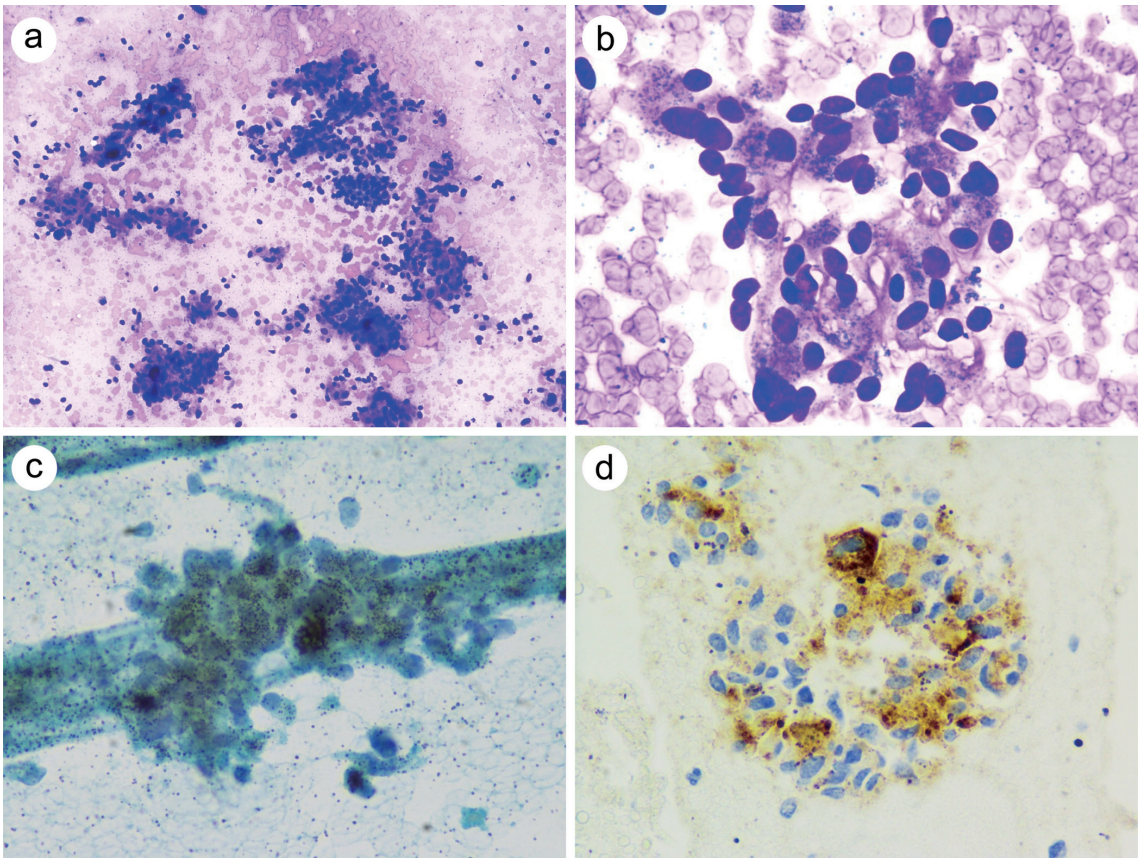

Fig. 1. Pigmented variant of pancreatic neuroendocrine tumors. (a) Cellular aspirate consists of loosely cohesive clusters of cells, and isolated cells or stripped nuclei (Romanowsky stain, 100×). (b and c) The cells present a bland, round-to-oval and eccentric nuclei, a finely granular chromatin, and an inconspicuous nucleoli. Abundant brown-black pigments are present in the cytoplasm and background (b: Romanowsky stain, 400×; c: Papanicolaou stain, 400×). (d) The cells are immunoreactive for chromogranin (Immunoperoxidase stain, 400×).

istry study can reliably distinguish these two entities from each other. PanNETs are immunoreactive for cytokeratins and neuroendocrine markers (synaptophysin, chromogranin, CD56 and INSM1), but are negative for melanocytic markers (S100, HMB45, Melan A and SOX10). Melanomas exhibit an opposite immunoprofile.

Pleomorphic variant

Occasional atypical cells with enlarged nuclei, known as endocrine atypia, can be observed in classic PanNETs. The pleomorphic variant exhibits striking anisonucleosis, and frequently contains cells with markedly enlarged and hyperchromatic nuclei in the background of other cells, presenting a more classic neuroendocrine tumor cytomorphology (Fig. 2). The chromatin in bizarre nuclei presents a smudgy and degenerative appearance, mimicking that observed in ancient schwannomas and uterine leiomyomas with bizarre nuclei (symplastic leiomyoma). This variant has recently been found to carry less aggressive clinical behaviors, when compared to classic PanNETs.[14] However, the extreme nuclear pleomorphism can render this variant prone to be misdiagnosed as adenocarcinoma.[11] Although the neuroendocrine origin of this variant can be confirmed by immunohistochemistry, care must be given to prevent the misclassification of this variant as a higher-grade tumor, such as neuroendocrine carcinoma. It is noteworthy that the tumor cell proliferative rate evaluated by the mitotic activity or Ki-67 labeling index can be utilized to grade PanNETs, and that the degree of nuclear atypia alone does not predict the prognosis. Neuroendocrine carcinoma demonstrates distinct genetic abnormalities, in addition to the high proliferative rate.[17]

Clear cell/lipid-rich variant

The terminologies "clear cell" variant and "lipid-rich" variant have been interchangeably used in most literatures to describe the seemingly similar morphologic variant of neuroendocrine tumors (NETs).[18–20] This variant of PanNETs can be sporadic or syndromic, and some cases have been identified in patients with von Hippel-Lindau syndrome (VHL) or multiple endocrine neoplasia type 1 syndrome (MEN I).[7,8,18,21,22] Neoplastic cells are morphologically characterized by the abundant clear-to-prominently vacuolated and bubbly cytoplasm (Fig. 3), and this is probably due to the cytoplasmic accumulation of lipid droplets.[8] These cells usually retain the nuclear features of classic PanNETs on FNA direct smears.[20–24]

The most important differential diagnosis of this variant is clear cell renal cell carcinoma (CCRCC), particularly because patients with VHL have an increased risk for development of CCRCC,[25] and because CCRCC is the most common metastatic malignancy in the pancreas. This variant should also be distinguished from ectopic adrenal tissues in the pancreas or adrenocortical neoplasm, among others.

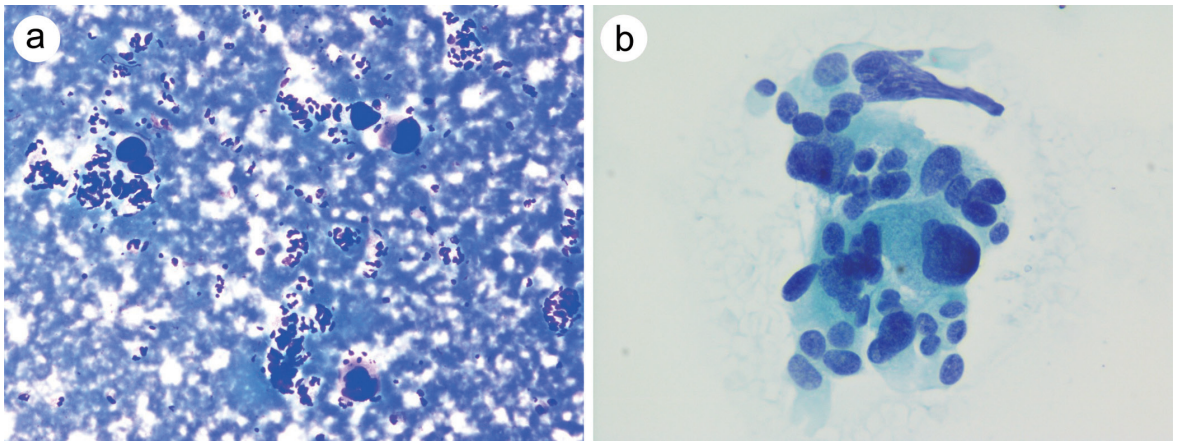

Fig. 2. Pleomorphic variant of pancreatic neuroendocrine tumors. (a) In the background of cells with conventional morphology, scattered bizarre cells are present with striking nuclear pleomorphism and hyperchromasia (Romanowsky stain, 100×). (b) The cells present a bizarre nuclei with irregular nuclear contours and hyperchromasia, but with a smudgy/degenerative chromatin (Papanicolaou stain, 400×).

Due to the significant cytomorphologic overlapping among the three entities, the combination of immunohistochemical stains would be crucial for making the distinctions. Typically, PanNETs are immunoreactive for cytokeratins and neuroendocrine markers. CCRCC is immunoreactive for cytokeratins and renal cell carcinoma markers (PAX8, RCC, CD10, vimentin and CA-IX). Adrenocortical tissues and neoplasms are immunoreactive for inhibin and SF1, but negative for cytokeratins. However, there are a few caveats when interpreting the immunohistochemical results in this regard. PAX8 is frequently expressed in PanNETs, when compared to NETs that originate from other anatomic sites.[26,27] Theoretically, the clear cell/lipid-rich variant of PanNETs can express PAX8. In addition, inhibin positivity has been reported

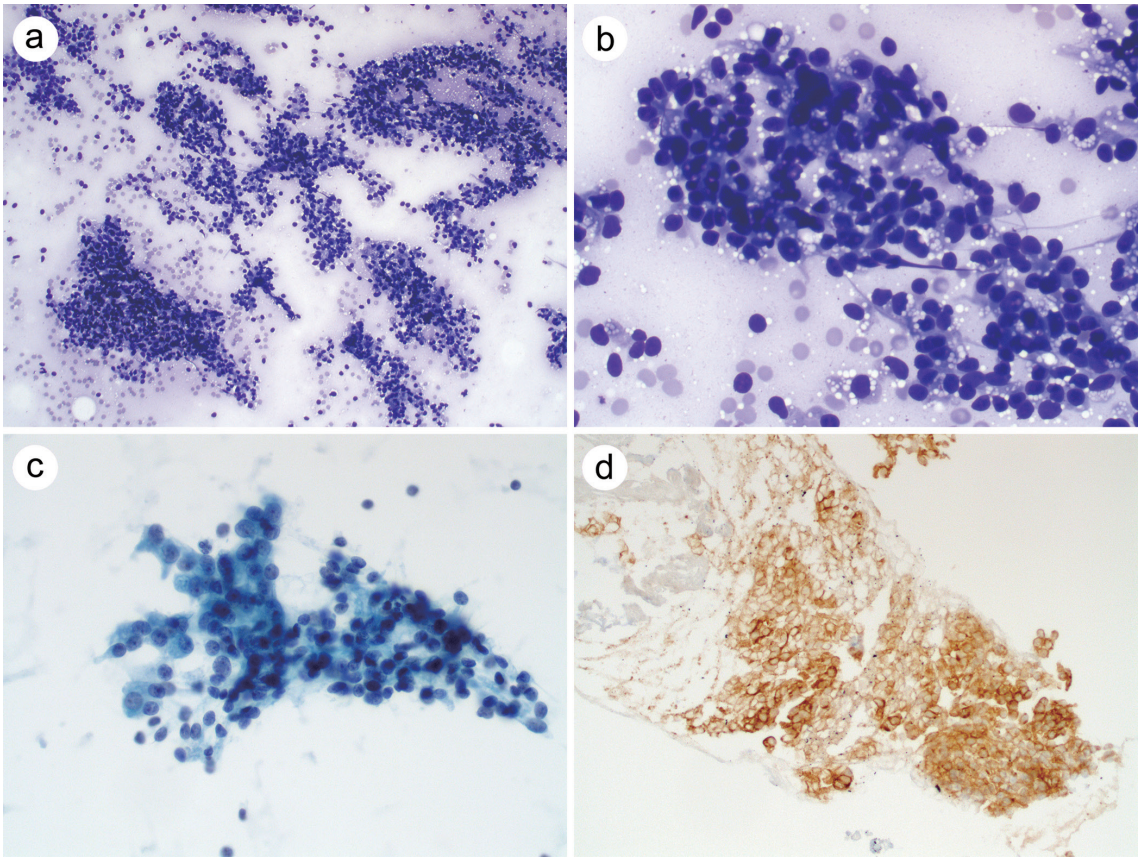

Fig. 3. Clear cell/lipid-rich variant of pancreatic neuroendocrine tumors. (a) Cellular aspirate consists of loosely cohesive clusters of cells and single cells (Romanowsky stain, 100×). (b) The cytoplasm contains prominent fine vacuoles (Romanowsky stain, 400×). (c) The nuclei are round to oval, with speckled chromatin and small nucleoli (Papanicolaou stain, 400×). (d) The cells are positive for synaptophysin (Immunoperoxidase stain, 400×).

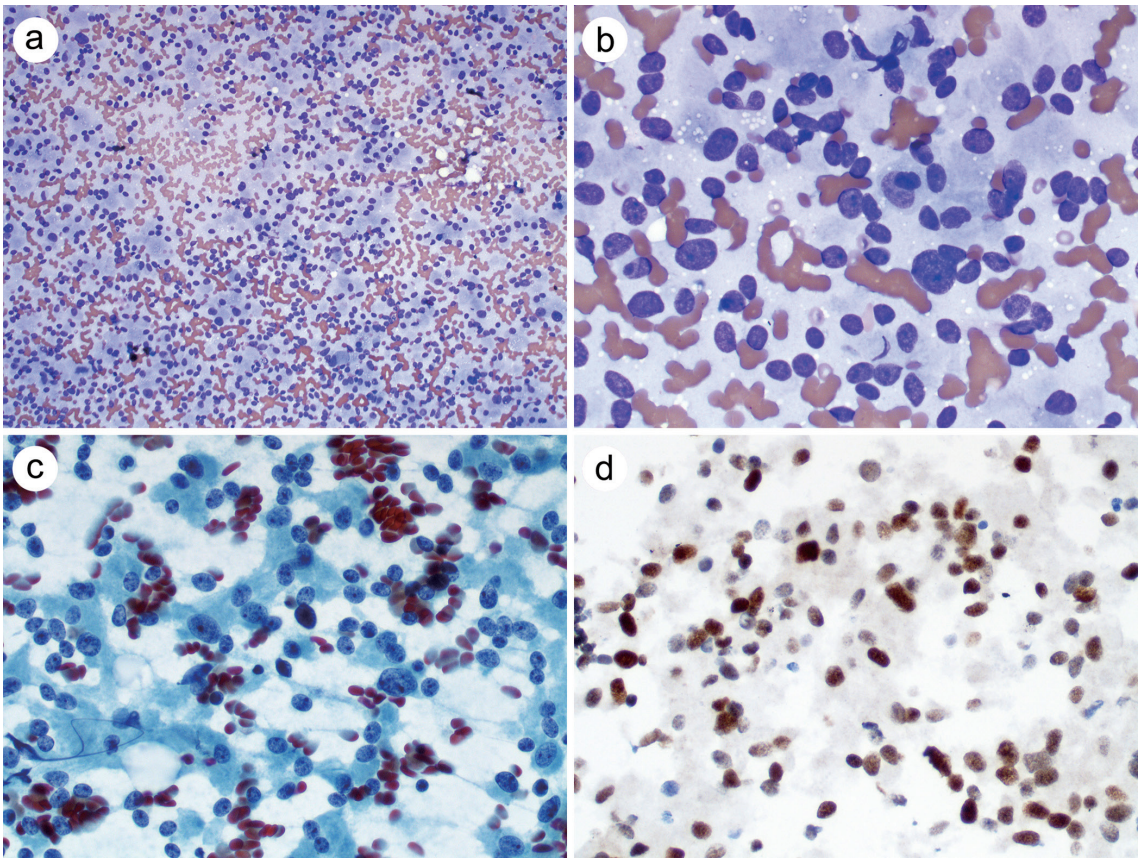

Fig. 4. Oncocytic variant of pancreatic neuroendocrine tumors. (a) Cellular aspirate consists of loosely cohesive clusters of cells and single cells (Romanowsky stain, 100×). (b) The cells have abundant granular cytoplasms (Romanowsky stain, 400×). (c) The nuclei are round-to-oval, with speckled chromatin and prominent nucleoli (Papanicolaou stain, 400×). (d) The cells are positive for INSM1 (Immunoperoxidase stain, 400×).

in clear cell/lipid-rich PanNETs,[22,24] while a significant proportion of adrenocortical tissues or neoplasms is immunoreactive for synaptophysin. Therefore, cytokeratin and a panel of at least two markers of each origin should be included during the diagnostic work-up.

Oncocytic variant

In comparison with classic PanNETs, cells in the oncocytic variant are arranged in more cohesive clusters on cytologic smears, contain abundant granular eosinophilic cytoplasm, and exhibit a greater degree of nuclear atypia, including nuclear pleomorphism, nuclear overlapping and prominent nucleoli, but usually maintain relatively smooth nuclear membrane and finely granular chromatin (Fig. 4). Conspicuous nuclear atypia can lead to the misdiagnosis of this variant as pancreatic ductal adenocarcinoma on cytology specimens.[28] This variant should be differentiated from intraductal oncocytic papillary neoplasm, due to the presence of abundant granular cytoplasm, especially when the PanNET is cystic.[29] Another important differential diagnosis is hepatocellular carcinoma, particularly when the tumor presents as a metastasis in the liver.[30] However, with the awareness of this variant, followed by judicious immunohistochemical work-up, arriving at a correct diagnosis would not be difficult. This variant is clinically more aggressive, and more frequently presents with a larger tumor size, a higher tumor grade, and a higher tumor stage when diagnosed.[14]

Conclusions

The pigmented, pleomorphic, clear cell and oncocytic variants of PanNETs have unique cytomorphologic features, but may share similar features with other neoplasms that are included in the differential diagnosis, as summarized in Table 1. Clinically, some variants are more aggressive, while other variants are more indolent. Recognizing the cytomorphologic and immunophenotypic features of these variants in FNA specimens is critically important to make a correct diagnosis, preventing diagnostic pitfalls, and guiding the appropriate clinical management.

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Conflict of interest

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Table 1. The cytomorphologic features and differential diagnosis of morphologic variants of well-differentiated pancreatic neuroendocrine tumors

	Cytomorphologic features	Differential diagnosis	Immunohistochemistry
Pigmented Variant	Relatively uniform cells, "salt and pepper" chromatin, black-brown pigments	Malignant melanoma	Malignant melanoma: (+) Melanocytic markers; (−) CKs, NE markers
Pleomorphic Variant	Bizarre cells with smudgy chromatin, classic NET cells in the background	Neuroendocrine carcinoma	Neuroendocrine carcinoma: (+) NE markers; Higher Ki-67 index
		Ductal adenocarcinoma	Ductal adenocarcinoma: (−) NE markers
Clear cell/lipid-rich Variant	Abundant clear to vacuolated and bubbly cytoplasm, "salt and pepper" chromatin	Clear cell renal cell carcinoma	Clear cell renal cell carcinoma: (+) RCC markers; (−) NE markers, SF1, inhibin
		Adrenal cortical neoplasm	Adrenal cortical neoplasm: (+) SF1, inhibin; (−) CKs, PAX8, CA-IX, RCC; (+/−) Synaptophysin (other NE markers negative)
Oncocytic Variant	Clear to vacuolated cytoplasm, larger nuclei, variable nucleoli	Ductal adenocarcinoma	Ductal adenocarcinoma: (−) NE markers
		Intraductal oncocytic papillary neoplasm	Intraductal oncocytic papillary neoplasm: (−) NE markers
		Hepatocellular carcinoma	Hepatocellular carcinoma:(+) HCC markers; (−) NE markers

CKs, cytokeratins; Neuroendocrine (NE) markers: synaptophysin, chromogranin, CD56 and INSM1; Melanocytic markers: S100, HMB45, Melan A, SOX10; Renal cell carcinoma (RCC) markers: PAX8, RCC, CD10, vimentin and CA-IX; Hepatocellular carcinoma (HCC) markers: HepPar1, arginase and glypican-3.

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Author contributions

Study concept and design: DL, HX and GC; Acquisition of data: DL, HX and GC; Analysis and interpretation of data: DL, HX and GC; Drafting of the manuscript: DL; Critical revision of the manuscript for important intellectual content: HX and GC; Administrative, technical, or material support, and study supervision: DL and GC. All authors made a significant contribution to the study, and approved the final manuscript.

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