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Clusterin in Neuroendocrine Epithelial Neoplasms: Absence of Expression in a Well-Differentiated Tumor Suggests a Jejunoileal Origin

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Abstract

Clusterin, a widely expressed, tissue-specific glycoprotein, is a diagnostic marker of several tumor types, including anaplastic large cell lymphoma, follicular dendritic cell sarcoma, and tenosynovial giant cell tumor. A recent study has suggested it is highly expressed by well-differentiated neuroendocrine tumors (NET) arising at most anatomic sites, with the exception of jejunoileal tumors, and that it is similarly not expressed by poorly differentiated neuroendocrine carcinomas (NEC). We sought to validate this result in a large cohort of NETs and NECs. Clusterin immunohistochemistry was performed on tissue microarrays of 255 NETs [45 lung, 4 stomach, 8 duodenum, 75 pancreas (62 primary, 13 metastatic), 107 jejunoileum (69 primary, 38 metastatic), 16 appendix] and 88 NECs (43 visceral, 45 Merkel cell). Extent (%) and intensity (0, 1+, 2+, 3+) of staining were assessed and an H-score (extent x intensity) calculated. An average H-score >5 was considered positive. Clusterin expression was noted in 82.4% of 148 non-jejunoileal NETs (average H-score 183) and only 8.4% of 107 jejunoileal NETs (average H-score 31), as well as 19.3% of NECs (average H-score 36). Clusterin is frequently, strongly expressed by NETs of diverse anatomic sites, with the exception of jejunoileal tumors, in which it is only rarely,

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AMB designed the study; JLH's laboratory performed the immunohistochemical staining; AMB, TWC, KMS, and JEM performed the research; AMB analyzed the data; AMB wrote the paper; TMO and JRH contributed patients; all authors edited the paper.

weakly expressed. It is occasionally, weakly expressed by NECs. Most metastatic NETs of occult origin arise in the pancreas or the jejunoileum. For cases in which an initial site of origin immunopanel (e.g., islet 1, PAX6, CDX2) is ambiguous, addition of clusterin may be diagnostically useful, with absence of expression suggesting a jejunoileal origin.

Keywords

Clusterin; immunohistochemistry; neuroendocrine; carcinoma of unknown primary; differential diagnosis

Introduction

The reported incidence of well-differentiated neuroendocrine tumors (NETs) has increased 5-fold over the last 30 years, and given relatively indolent disease biology, their population prevalence is greater than that of gastric, pancreatic, or esophageal cancer.(1) NETs arise at diverse anatomic sites, predominantly in the lung, tubal gut, and pancreas. Ten to twenty percent of tumors present as metastases of occult origin, and determining the site of origin is prognostically and therapeutically relevant.(2–4) Most NETs presenting as metastasis of unknown primary arise from the ileum or pancreas.(5–8) Immunohistochemistry is useful for assigning NET site of origin.(4) At the University of Iowa our primary panel includes islet 1, PAX6 (both for pancreas), and CDX2 (midgut). In our experience, up to 10% of tumors of pancreatic or jejunoileal origin are negative with our primary panel, and additional markers in this setting are of interest.(9)

Clusterin is a widely expressed, tissue-specific glycoprotein (Table 1) with numerous roles in health and disease. Named for its ability to aggregate Sertoli cells and erythrocytes, clusterin was independently discovered over a dozen times, reflecting its diverse biologic functions, which are generally cytoprotective (Table 2).(10–25) Fundamentally, it is a molecular chaperone, able to bind diverse molecules via hydrophobic interactions.(26, 27)

Clusterin came to prominence in diagnostic pathology in 2000, when gene expression profiling of 31 hematolymphoid neoplasm cell lines identified expression limited to anaplastic large cell lymphoma (ALCL).(28) It was subsequently independently validated as an ALCL marker.(29–31) It has also found use as a marker of follicular dendritic cell sarcoma,(32, 33) tenosynovial giant cell tumor,(34) large cell transformation in mycosis fungoides,(35) and, most recently, hepatocellular carcinoma.(36)

We read with interest a recent report by Mourra and colleagues describing clusterin expression in most NETs from diverse anatomic sites, except the jejunoileum in which expression was rare; the protein was also not expressed by poorly differentiated neuroendocrine carcinomas (NECs). (37) This was an extension of earlier work from the group, which had shown that clusterin was frequently expressed by pancreatic NETs but not by solid pseudopapillary neoplasm.(38) We sought to validate these results in a large cohort of NETs and NECs, in which case absence of clusterin expression in a metastatic NET of occult origin would suggest a jejunoileal origin.

Materials and methods

Neoplasms were identified from the surgical pathology archives of the University of Iowa Hospitals and Clinics. Original glass slides were reviewed, the diagnosis was confirmed, and a “best tumor block” was identified. Tissue microarrays (TMAs) were constructed using the Manual Tissue Arrayer MTA-1 (Beecher Instruments; Sun Prairie, WI), with the following 343 tumors arrayed as triplicate 1 mm cores: 255 NETs (45 lung, 4 stomach, 8 duodenum, 62 pancreas primary, 13 pancreas metastatic, 69 jejunoileum primary, 38 jejunoileum metastatic, 16 appendix) and 88 NECs (43 visceral, 45 Merkel cell).

Clusterin immunohistochemistry was performed manually on 4-μm-thick tissue sections after deparaffinization, rehydration, and pressure cooker heat-induced epitope retrieval in Target Retrieval Solution (pH 6.1; Dako; Carpinteria, CA) using a mouse monoclonal antibody directed against the α-chain (clone 41D; 1:20,000 dilution; EMD Millipore; Temecula, CA) and the polymer-based Dako EnVision+ detection system. Palatine tonsil served as the positive (follicular dendritic cells, squamous epithelium, and endothelium) and negative (lymphocytes) external control.

Clusterin expression was evaluated for extent (0–100%) and intensity (0–3+) in each TMA core and an overall H-score (mean extent*intensity) was calculated for each neoplasm. An average H-score >5 was considered positive. This research was conducted with University of Iowa Institutional Review Board approval.

Results

Clusterin expression was noted in 82.4% of 148 non-jejunoileal NETs (average positive H-score 183) (Figure 1a–b) and only 8.4% of 107 jejunoileal NETs (average positive H-score 31) (Figure 1c–f), as well as 19.3% of NECs (average positive H-score 36) (see Figure 2a–d). We also observed strong expression in non-neoplastic islets, which predominated at the periphery (Figure 3a). There was moderately strong staining in the Paneth cells of ileal mucosa, but enteroendocrine cells appeared negative (Figure 3b). Detailed expression data are presented in Tables 3 and 4.

Discussion

We detected clusterin expression in the vast majority (82.4%) of non-jejunoileal NETs, with expression typically strong (average positive H-score 183). Notably this included 89% of 62 pancreatic primaries and 100% of 13 pancreatic metastases (average positive H-score 215 in both of these settings). This contrasted with infrequent, weak expression in jejunoileal tumors (6% of 69 primaries and 13% of 38 metastases with average positive H-scores of 11 and 50, respectively). We also found weak clusterin expression in 20% of NECs, with similar frequencies in visceral and cutaneous primaries.

Our results are nearly identical to those of Mourra and colleagues, who found clusterin expression in 85% of 80 non-jejunoileal and 8% of 51 jejunoileal NETs.(37, 38) In one of these studies they also found no expression in 13 WHO G3 tumors.(37) In the only other study of clusterin expression in neuroendocrine epithelial neoplasms, which was limited to

the pancreas, Henderson-Jackson et al reported staining in 92% of 59 neoplasms, with strong staining in 61%.(39) Six of the neoplasms in this study were G3. Specific clusterin results were not presented for the G1–G2 vs G3 neoplasms, and, based on our results, it is likely that the overall positive and strong positive rates in their NETs are greater than those reported for the entire cohort.

Among the myriad contexts in which it was independently discovered, clusterin (initially known as glycoprotein III in this setting) was found to be associated with the membrane and soluble contents of adrenal chromaffin granules.(12, 40, 41) Clusterin was subsequently identified as a component of the large dense core secretory vesicles of thyroid parafollicular cells, anterior and posterior pituitary, and parathyroid; in these endocrine tissues, expression is limited to secretory vesicles.(41–43) Clusterin expression has also been described in the developing rat and porcine endocrine pancreas, with initially widespread distribution in fetal pancreas becoming ultimately restricted to a subset of glucagon-expressing cells in the adult.(44–46) We similarly detected clusterin expression in non-neoplastic islets, strongest at the islet periphery, where glucagon-producing alpha cells reside. Of note, though Aronow and colleagues, in their detailed anatomic description of clusterin expression described strong expression in pancreatic acinar cells and no expression in pancreatic islets, review of the relevant image from this manuscript demonstrates moderately strong expression in islets and not in acinar parenchyma.(47) In colonic mucosa, Andersen and colleagues found clusterin expression limited to a subset of the total enteroendocrine cell population; in this same report, a colonic NET also showed strong expression.(48)

Tumors recapitulate normal cell types. NETs variously recapitulate the neuroendocrine cell types native to the anatomic site at which they arise (e.g., enterochromaffin-like cells in the body of the stomach, alpha or beta cells in the pancreas, L cells in the rectum). Jejunoileal NETs recapitulate serotonin-producing, enterochromaffin (EC) cells. We hypothesize that most NETs express clusterin because clusterin is expressed by these tumors' cognate normal cell types. Given clusterin's function as a molecular chaperone, one would speculate a role in the secretion of peptide hormones/biogenic amines from chromaffin granules/dense core secretory vesicles, possibly involving the loading or recycling of these organelles.

As opposed to expression in non-neoplastic islets, we failed to demonstrate significant clusterin expression in the neuroendocrine cells of non-neoplastic ileum. Among neuroendocrine cells, EC cells are unique in the small amount of hormone that is released with each exocytosis event (i.e., 70x less than is released by chromaffin cells), which is more akin to that seen at synapses.(49) EC cell hormone-release kinetics are attributed to a smaller fusion pore size and may be due to a preference for "kiss-and-run" rather than full vesicle fusion.(50) The unique biology of the EC cell should be reflected in its structure, and lack of clusterin expression appears to be at least a marker of this phenotype.

Clusterin has been shown to function as a tumor suppressor and an oncoprotein.(51–53) Clusterin is known to inhibit NF-κB signaling, resist the epithelial-to-mesenchymal transition, and is typically down-regulated in cancer relative to normal tissue. On the other hand, clusterin is known to be upregulated in cancers resistant to chemo-, hormone, or radiotherapy, probably related to the protein's anti-apoptotic function. Custirsen (OGX-111)

is an antisense oligonucleotide to clusterin that has made it to phase III clinical trials (in combination with conventional chemotherapy) in metastatic castration-resistant prostate cancer and metastatic non-small cell lung cancer.(53–56) Given frequent, strong expression in non-jejunoileal NETs, a clinical trial in this setting (e.g., in advanced tumors progressing on somatostatin analogue therapy) could be considered.

Conclusion

We found frequent, strong clusterin expression in non-jejunoileal and only rare, weak expression in jejunoileal NETs. Clusterin is known to be expressed by chromaffin granules and dense core secretory vesicles of neuroendocrine cells, and we speculate that lack of expression in jejunoileal tumors may reflect the unique character of jejunoileal EC cells. We frequently encounter metastatic NETs of occult origin and perform a primary panel of immunostains including islet 1, PAX6, and CDX2 to assist in assigning site of origin. Given our results here, we have added clusterin to the second tier of our site of origin algorithm, applied when the initial panel is negative (Figure 4). Clusterin provides the additional advantage of multiple potential diagnostic uses (e.g., in ALCL, follicular dendritic sarcoma, and tenosynovial giant cell tumor). Finally, given frequent, strong expression, clusterin represents a logical therapeutic target in advanced NETs, in which custirsen would be expected to act as a chemo- and/or radiosensitizer.

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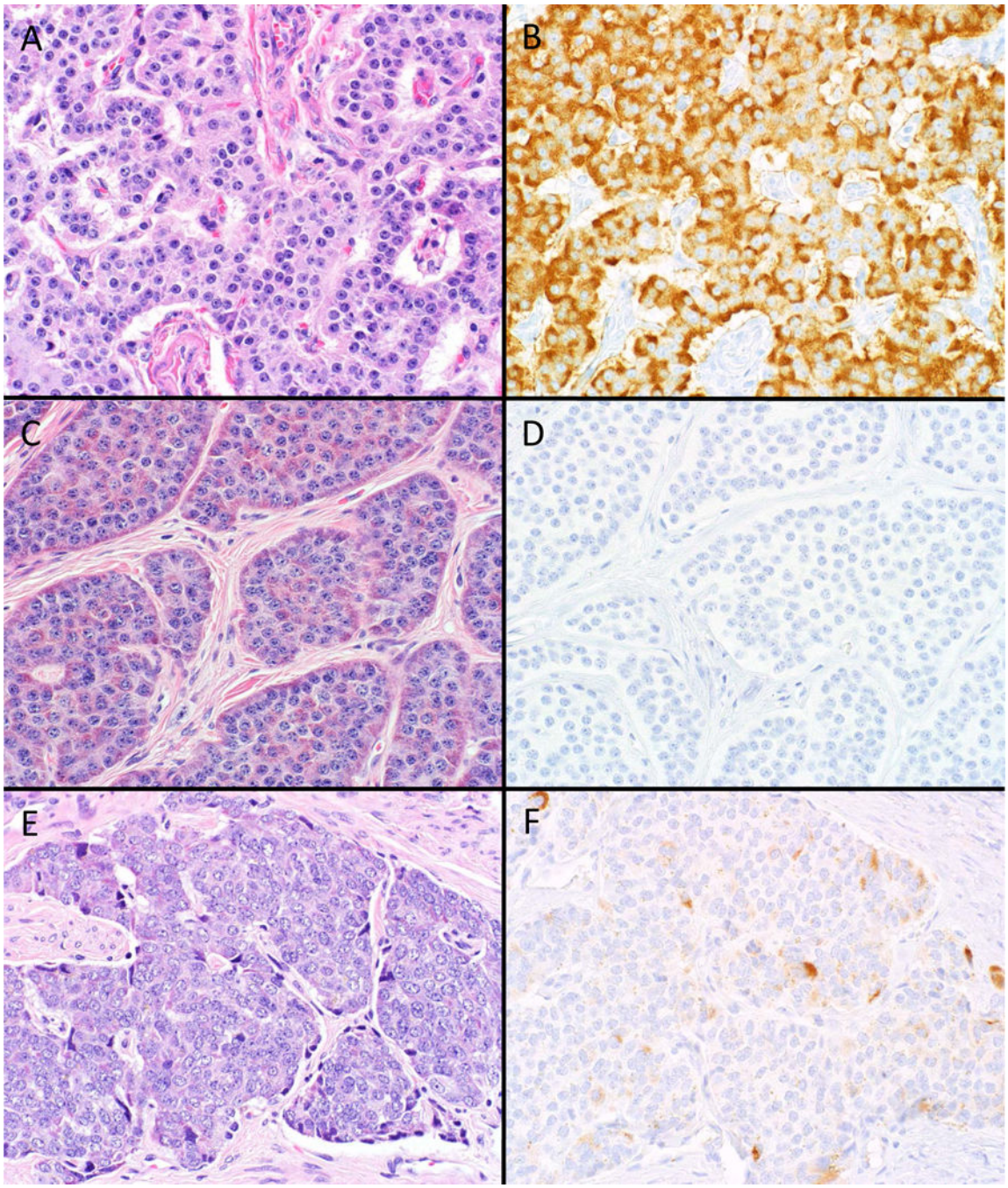

Figure 1.

A–F, Clusterin Staining in Well-Differentiated Neuroendocrine Tumors (NET). The vast majority of non-jejunoileal NETs (pancreatic primary depicted in A) demonstrate strong clusterin expression (B); most jejunoileal tumors (C) are entirely negative (D), while occasional jejunoileal tumors (E) demonstrate weak, patchy staining (F) (original magnification of each image 400×).

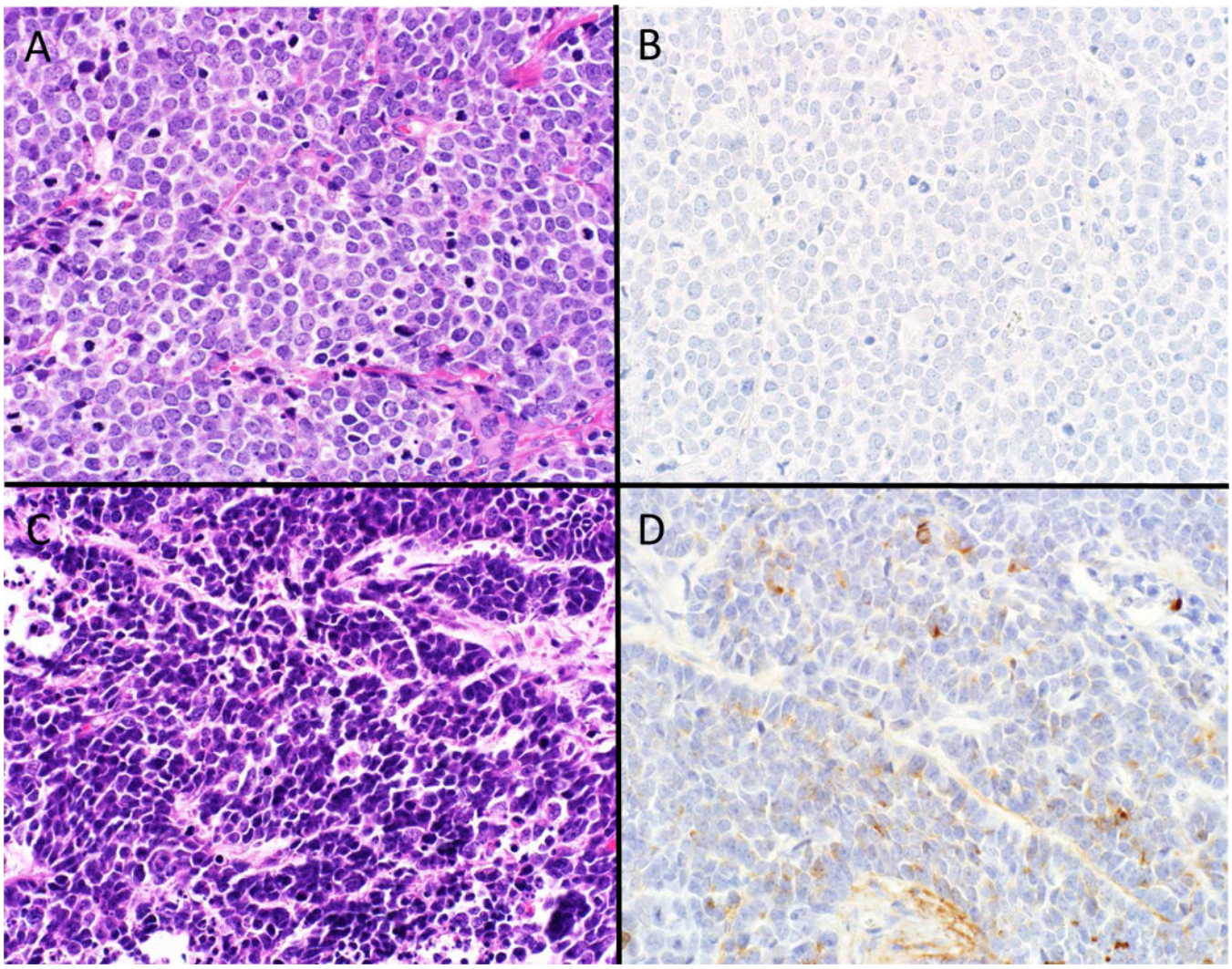

Figure 2.

A–D, Clusterin Staining in Poorly Differentiated Neuroendocrine Carcinomas (NEC). A majority of NECs (Merkel cell carcinoma depicted in A) do not express clusterin (B), while up to 20% (visceral small cell carcinoma depicted in B) show weak, patchy staining (D) (original magnification of each image 400×).

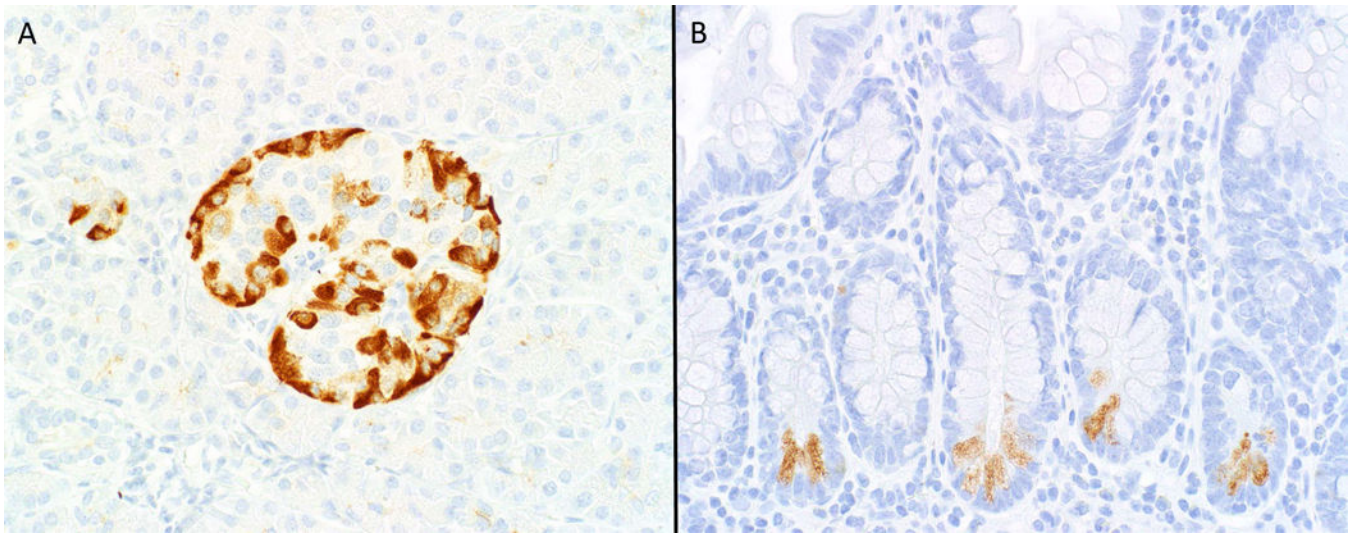

Figure 3.

A–B, Clusterin Staining in Cognate Normal Tissues. Clusterin is strongly expressed by non-neoplastic islets, with expression predominantly at the periphery, where glucagon-expressing alpha cells reside (A); In non-neoplastic ileal mucosa moderately strong staining is noted in Paneth cells (readily identified by their location in crypt bases and luminal orientation of cytoplasmic granules), while no staining is noted in enteroendocrine cells (which also reside in crypt bases but with abluminal orientation of cytoplasmic granules) (B) (original magnification of both images 400×).

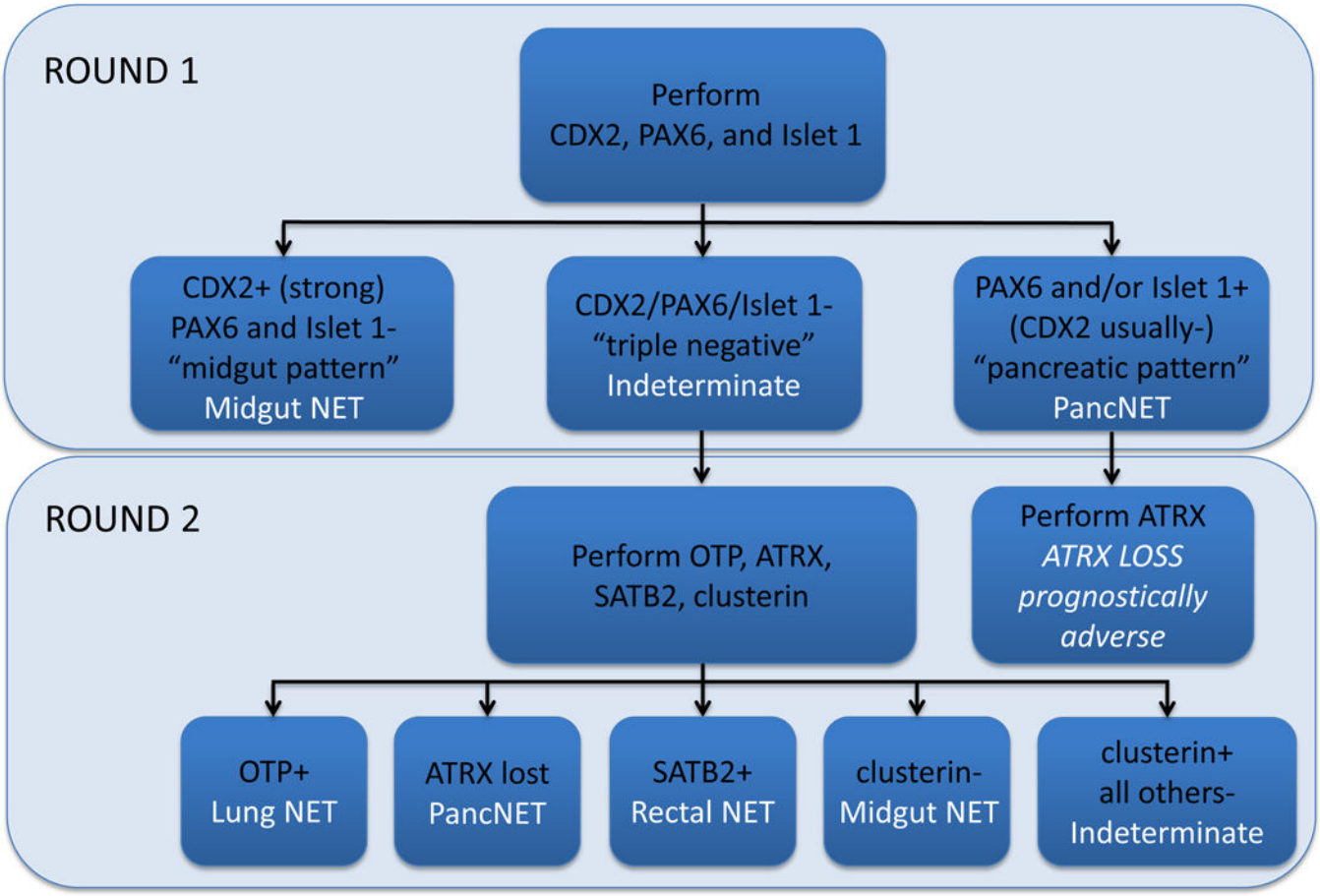

Figure 4.

Iowa Well-Differentiated Neuroendocrine Tumor Site of Origin Immunohistochemical Classifier. Clusterin immunohistochemistry is potentially useful in tumors considered "indeterminate" for site of origin on initial islet 1/PAX6/CDX2 staining. In this context, lack of clusterin staining would support a jejunoileal origin.

Table 1

Distribution of Clusterin Expression

Organ	Cell Type
Esophagus	Squamous epithelium (excepting basal cells)
Stomach	Foveolar epithelium (proximal stomach) Chief cells Antral glands
Duodenum	Brunner glands
Liver	Hepatocytes Bile duct epithelium
Pancreatobiliary tree	Pancreatic ductal epithelium Islets of Langerhans * Extrahepatic biliary epithelium Gallbladder epithelium
Testis	Sertoli cells
Epididymis	Epididymal epithelium
Ovary	Granulosa cells
Uterus	Endometrial epithelium Endocervical epithelium Ectocervical epithelium
Vagina	Squamous epithelium
Kidney	Distal convoluted tubules Transitional epithelium of pelvicalyceal system
Ureter	Transitional epithelium
Bladder	Transitional epithelium
Adrenal cortex	Zona glomerulosa
Larynx/trachea	Submucosal glands
Heart	Myocytes of left and right atria
Brain	Choroid plexus Ependyma Subpopulation of neurons and glia
Eye	Ciliary body epithelium
Joint space	Synovial lining cells

Based on in situ hybridization data from: Aronow BJ, Lund SD, Brown TL, Harmony JA, Witte DP. Apolipoprotein J expression at fluid-tissue interfaces: potential role in barrier cytoprotection. *Proceedings of the National Academy of Sciences of the United States of America*. 1993;90(2): 725–9.

* See Discussion

Table 2

Settings in which Clusterin was Independently Discovered

Study	Name Assigned	Comment
Fischer-Colbrie et al (1982)	Glycoprotein III	Membrane-bound and soluble glycoprotein of chromaffin granules
Blaschuk et al (1983)	Clusterin	Isolated from ram rete testis fluid based on ability to aggregate Sertoli cells and erythrocytes; secreted by Sertoli cells
Collard and Griswold (1987)	Sulfated glycoprotein 2 (SGP-2)	Primary Sertoli cell secretory protein; binds to acrosome and distal tail of spermatozoa
Léger et al (1987)	Testosterone-repressed prostate message (TRPM-2)	Most upregulated ventral prostate protein upon castration (i.e., androgen withdrawal)
Michel et al (1989)	T64	Most abundant mRNA in Rous sarcoma virus-transformed quail neuroretinal cells
Kirszbaum et al (1989)	Serum protein 40 kD,40kD (SP-40,40)	Member of the SC5b-9 complement complex (i.e., soluble terminal complement complex)
Jenne and Tschopp (1989)	Complement cytolysis inhibitor (CLI)	Inhibitor of SC5b-9 complex
de Silva et al (1990)	Apolipoprotein J (apoJ)	High density lipoprotein (HDL)-associated glycoprotein
May et al (1990)	Alzheimer's disease hippocampus clone 9 (p-ADHC-9)	Increased mRNA expression in Alzheimer's disease hippocampus
Hartmann et al (1991)	80-kD glycoprotein (gp 80)	Glycoprotein secreted from the apical surface of Madin-Darby canine kidney (MDCK) cell line
James et al (1991)	NA1/NA2	High density lipoprotein (HDL)-associated glycoprotein
Danik et al (1991)	pTB16	Increased mRNA expression in high-grade astrocytoma
Jones et al (1992)	K611	Increased mRNA expression in retinitis pigmentosa retinas
Diemer et al (1992)	38-kD protein (pc38K)	Increased protein expression in differentiating (i.e., nodular) smooth muscle cells in culture

Modified from Table in: Rosenberg ME, Silkensen J. Clusterin: physiologic and pathophysiologic considerations. *Int J Biochem Cell Biol.* 1995;27(7):633–45.

Table 3

Clusterin Expression in Well-Differentiated Neuroendocrine Tumors

Anatomic Site	n	% Positive	Average H-score (if positive)
Lung	45	73.3	141
Stomach	4	50	165
Duodenum	8	62.5	224
Pancreas Primary	62	88.7	215
Pancreas Metastasis	13	100	215
Jejunoileum Primary	69	5.8	11
Jejunoileum Metastasis	38	13.2	50
Appendix	16	93.8	138

Table 4

Clusterin Expression in Poorly Differentiated Neuroendocrine Carcinomas

Tumor Type	n	% Positive	Average H-score (if positive)
Small Cell Carcinoma	43	21	51
Merkel Cell Carcinoma	45	18	22