Published in final edited form as:

Horm Cancer. 2013 June ; 4(3): 165–175. doi:10.1007/s12672-013-0134-1.

Altered *PTEN*, *ATRX*, *CHGA*, *CHGB* & *TP53* Expression are Associated with Aggressive VHL-Associated Pancreatic Neuroendocrine Tumors

Allison B. Weisbrod, MD, MPH¹, Lisa Zhang, PhD¹, Meenu Jain, PhD¹, Stephanie Barak, MD², Martha M. Quezado, MD², and Electron Kebebew, MD¹

¹Endocrine Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

²Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Abstract

Von Hippel-Lindau (VHL) syndrome is an inherited cancer syndrome in which 8-17% of germline mutation carriers develop pancreatic neuroendocrine tumors (PNETs). There is limited data on prognostic markers for PNETs other than Ki-67, which is included in the World Health Organization classification system. Recently, specific genes and pathways have been identified by whole exome sequencing which may be involved in the tumorigenesis of PNETs and may be markers of disease aggressiveness. The objective of this study was to identify molecular markers of aggressive disease in VHL-associated PNETs. The protein expression of 8 genes (*PTEN*, *CHGA*, *CHGB*, *ATRX*, *DAXX*, *CC-3*, *VEGF*, *TP53*) was analyzed in PNETs by immunohistochemistry and compared to clinical data, VHL genotype, functional imaging results, and pathologic findings. Subcellular distribution of PTEN, CHGA and ATRX were significantly different by WHO classifications ($p < 0.05$). There was decreased PTEN nuclear to cytoplasmic ratio ($p < 0.01$) and decreased CHGA nuclear expression ($p = 0.03$) in malignant samples as compared to benign. Lower cytoplasmic CHGB expression ($p = 0.03$) was associated with malignant tumors and metastasis. Higher nuclear expression of PTEN was associated with *VHL* mutations in exon 3 ($p = 0.04$). Higher PTEN and CHGB expression was associated with higher FDG-PET avidity ($p < 0.05$). Cytoplasmic expression of CC-3 was associated with higher serum Chromogranin A levels ($\sigma = 0.72$, $p = 0.02$). Lastly, greater cytoplasmic expression of p53 was associated with metastasis. Our findings suggest that altered PTEN, ATRX, CHGA and CHGB expression are associated with aggressive PNET phenotype in VHL and may serve as useful adjunct prognostic markers to Ki-67 in PNETs.

Keywords

PTEN; ATRX; DAXX; CHGA; CHGB; CC-3; VEGF; TP53 Von Hippel-Lindau Syndrome; Immunohistochemistry; Pancreatic Neuroendocrine Tumor; Neoplasm

Corresponding Author: Electron Kebebew, MD 10 Center Drive Bethesda, MD 20892 kebebewe@mail.nih.gov Tel: 301-594-6269 FAX: 301-480-0599.

Disclosure Statement: The authors have nothing to disclose.

Introduction

Von Hippel-Lindau (VHL) syndrome is an inherited cancer syndrome due to a germline inactivating mutation in the *VHL* tumor suppressor gene. Patients with VHL have a high-risk of developing multiple neoplasms, including an 8-17% risk of developing pancreatic neuroendocrine tumors (PNETs). These tumors may be benign or malignant, and can be synchronous or metachronous.(1, 2)

Several prognostic classification systems (World Health Organization (WHO), European Neuroendocrine Tumor Society (ENETS), and American Joint Committee on Cancer (AJCC)) for PNETs have been developed.(3-5) While these classification systems have been validated as a risk stratification tool (5-9) there has been little additional prognostic information available for PNETs and currently, only Ki-67 has been used as a tissue marker.(10)

A recent study addressed this gap in PNET prognostication and sought to identify the most frequently mutated genes in sporadic PNETs. The investigators used exome sequencing in 10 sporadic PNETs, with additional screening for the most common mutations found in an additional 58 samples.(10) Jiao and colleagues identified the presence of somatic mutations in *MEN1* (44.1%), *DAXX* (25%), *ATRX* (17.6%) and *PTEN* (7.3%). Furthermore, the presence of somatic mutations in *MEN1* and/or *DAXX/ATRX* was associated with better survival as compared to patients with PNETs which had wild type *MEN1* and/or *DAXX/ATRX*.(10)

Several candidate markers have also been associated with disease aggressiveness. The expression of *CHGA* and *CHGB* varies among different types of neuroendocrine tumors (NETs). In general, greater expression of *CHGA* is seen in well-differentiated as opposed to poorly differentiated NETs.(11) Increased *CHGB* expression is associated with a favorable prognosis in patients with sporadic medullary thyroid cancer, but inversely in patients with prostate cancer.(11) Higher expression of the apoptosis related protein, cleaved-caspase 3 (CC3), has been associated with prognosis in PNETs with greater expression in benign PNETs as compared to malignant sporadic PNETs.(12) The expression of vascular endothelial growth factor (VEGF) has been associated with tumor aggressiveness and patient prognosis in a variety of human malignancies.(13) Lastly, altered p53 expression is frequently seen in many tumors, with TP53 cited as the most frequently mutated gene associated with malignancy.(14) Yachida, et al, recently described abnormal immunolabeling of 9 of 19 poorly differentiated PNETs, but 0 of 11 well-differentiated PNETs.(15)

To our knowledge, the protein expression of these genes has not been examined in the context of VHL-associated PNETs and the utility of the WHO, ENET and AJCC prognostic scoring systems in VHL-associated PNETs is not known. Thus, the aim of this study was to determine if any of the genes were associated with PNET phenotype using immunohistochemistry analysis for PTEN, ATRX, DAXX, CHGA, CHGB, CC-3, VEGF and p53.

Materials and Methods

Patients and Tissue Samples

Patients with VHL syndrome who had a pancreatic manifestation of their disease were enrolled in a prospective protocol approved by the Office of Human Subject Research at the National Institutes of Health (NIH). All participants gave written informed consent. For all patients who met criteria for an operation, a portion of the surgical specimen was snap-

frozen at the time of surgery and stored at -80°C. A section of each specimen was fixed in formalin and embedded in paraffin. These samples included well-differentiated neuroendocrine tumors with benign characteristics (n=4), well-differentiated neuroendocrine tumors with uncertain characteristics (n=7), neuroendocrine carcinoma (n=4), microcystic serous cystadenoma (n=4) and normal pancreas (n=4). Demographic, clinical, radiologic and pathology data were collected and are summarized in Table 1.

Immunohistochemistry

Immunohistochemistry was performed using Vectastain ABC kit, (Vector Laboratories, Inc., Burlingame, California) according to the manufacturer's recommendations. Five micron sections of tissue samples were de-paraffinized for 30 minutes in xylene then rehydrated first for 30 minutes in 100% ethanol, followed by a 5 minute rinse each in 95%, 70% and 50% ethanol. Finally, tissue sections were washed for 5 minutes in de-ionized water. Rehydrated sections underwent a two-step antigen retrieval process, first incubated for 30 minutes at room temperature in 0.1% saponin (Calbiochem, Darmstadt, Germany), and then in 1× pH 6.0 citrate buffer (Thermo Scientific, Fremont, California) for 15 minutes at 100°C. Tissue sections were incubated for 20 hours with respective primary antibodies: PTEN rabbit monoclonal primary antibody against the carboxy-terminal sequence at a 1:100 dilution (#9559, Cell Signaling Technology, Inc., Danvers, Massachusetts); ATRX rabbit polyclonal primary antibody against amino acid sequence 2161-2413 at a 1:100 dilution (#S1800, Epitomics, Inc., Burlingame, California); DAXX rabbit polyclonal primary antibody against amino acid sequence 261-274 at a dilution of 0.9µg/mL (#NBP1-03157, Novus Biologicals, Littleton, CO); CHGA rabbit polyclonal primary antibody against a site within amino acid sequence 250-457 of P10645 at a 1:2200 dilution (ab15160, Abcam Inc, Cambridge, Massachusetts); CHGB rabbit polyclonal primary antibody against 15 amino acids in the C-terminal at a 1:2500 dilution (ab12242, Abcam Inc, Cambridge, Massachusetts); CC-3 rabbit polyclonal primary antibody against a 17/19kDa fragment resulting from cleavage adjacent to Asp175 at a 1:1400 dilution (#9661, Cell Signaling Technology, Inc., Danvers, Massachusetts); VEGF rabbit polyclonal primary antibody against isoforms 121, 145, 165, 189 and 206 at a 2.5µL/mL dilution (ab46154, Abcam Inc, Cambridge, Massachusetts); and p53 mouse monoclonal primary antibody against a site located between the N-terminal amino acids 1-45 at a 1:25 dilution (M7001, Dako North America, Inc., Carpinteria, California). DAB peroxidase substrate kit (Vector Laboratories, Inc.) was used for staining prior to dehydration of the tissue samples. Bronchial epithelial cells were used as a positive control for PTEN staining, gallbladder mucosa was used as a positive control for ATRX, CC-3 and p53 staining; normal pancreas acinar cells were used as a positive control for DAXX and normal pancreatic islet cells were used as a positive control for CHGA, CHGB and VEGF. Negative control tissue sections from each tumor sample were incubated for 20 hours with rabbit serum, rather than primary antibody.

Analyses

Each sample was scored by two independent evaluators for both nuclear and cytoplasmic intensity and percent of cells positive. A semi-quantitative scale was used. Intensity was scored as none (1), faint (2), moderate (3) or strong (4). Percent of positive cells was categorized as <33% (1), between 33% and 66% (2) and >66% (3). Independent scores were evaluated using Spearman's Correlation test prior to averaging for further analysis, with $\sigma > 0.7$ for all categories. The average nuclear intensity and nuclear distribution score for each sample were multiplied to create a total nuclear score (16). This was similarly performed to calculate a cytoplasmic score. Scores for nuclear and cytoplasmic expression of PTEN immunostaining as well as nuclear-to-cytoplasmic ratio were compared within each pathologic diagnosis. Grubb's outlier analysis was performed of each group, with significant outliers, if present, discarded prior to determining the average score for each

group. Average nuclear scores, cytoplasmic scores and nuclear-to-cytoplasmic ratios were compared amongst each histopathologic diagnosis and to patient clinical characteristics.

Results

Phosphatase and Tensin Homolog (PTEN)

All tissue samples showed positive immunostaining after incubation with anti-PTEN protein antibody. Inter-reader correlation was high ($\sigma = 0.7060$, $p < 0.01$). Representative images are shown in Figure 1. Both cytoplasmic expression of PTEN and nuclear-to-cytoplasmic (N/C) ratio of PTEN expression was significantly different by histopathologic diagnostic groups ($p < 0.05$ & $p < 0.02$, respectively). The N/C ratio was lowest for carcinomas ($p = 0.02$).

We observed no significant difference in PTEN immunostaining by patient age, gender, Ki-67 index, 6-L- ^{18}F -fluorodihydroxyphenylalanine (^{18}F -FDOPA) PET avidity or SUV value. However, patients with a *VHL* mutation in exon 3 had significantly higher nuclear expression of PTEN compared with patients with mutations in exon 1 or 2 ($p = 0.04$, Figure 2). PTEN cytoplasmic expression was higher in patients with F-18-Deoxyglucose Positron Emission Tomography (^{18}F -FDG PET) avid tumors ($p = 0.04$, Figure 3).

Alpha Thalassemia / Mental Retardation Syndrome X-Linked (ATRX)

All tissue samples showed positive immunostaining for ATRX. Inter-reader correlation was high ($\sigma = 0.84$, $p < 0.01$). Representative images are shown in Figure 4. Within categories of PNETs, the N/C ratio was significantly higher by WHO histologic classification ($p = 0.04$). No additional statistically significant associations were identified by clinical, radiographic or genotypic data.

Death-Associated Protein 6 (DAXX)

All tissue samples showed positive immunostaining for DAXX protein. Inter-reader correlation was high ($\sigma = 0.86$, $p < 0.01$). We found no statistically significant difference in tumor DAXX protein expression by pathologic diagnosis, clinical data, *VHL* exon mutation and functional imaging results.

Chromogranin A (CHGA)

Seventeen of twenty (85%) tumor tissue samples showed positive immunostaining for CHGA, with 1 of 4 MCA samples and 2 of 4 malignant samples showing no evidence of staining. Inter-reader correlation was high ($\sigma = 0.80$, $p < 0.01$). Representative images are shown in Figure 5. As tumor grade increased, nuclear expression of CHGA decreased ($p = 0.03$). Additionally, primary tumors from patients with evidence of metastatic disease demonstrated significantly less CHGA protein expression than tumors in patients without metastases ($p = 0.03$). There was no significant association between serum Chromogranin A values and tumor CHGA immunostaining.

Chromogranin B (CHGB)

All tissue samples, except one malignant tumor, showed positive immunostaining for CHGB. Inter-reader correlation was high ($\sigma = 0.83$, $p < 0.01$). Representative images are shown in Figure 6. Primary tumor samples from patients with metastatic disease had less cytoplasmic expression than tissue samples from patients without metastatic disease ($p = 0.03$). Additionally, if a cytoplasmic outlier sample was excluded (outlier score = 7.5, remaining average score = 1.5), WHO grade III tumors had lower cytoplasmic expression than MCAs and WHO grade I and II tumors.

Cleaved Caspase-3 (CC-3)

All tissue samples showed positive immunostaining for CC-3 protein. Inter-reader correlation was high ($\sigma = 0.73$, $p < 0.01$). There was a significant correlation between cytoplasmic expression of CC-3 and serum values of chromogranin A ($\sigma = 0.72$, $p = 0.02$).

Vascular Endothelial Growth Factor (VEGF)

All tissue samples showed positive immunostaining for VEGF protein. Inter-reader correlation was high ($\sigma = 0.74$, $p < 0.01$). No outliers were identified but we found no statistically significant associations with pathologic diagnosis, clinical data, VHL exon mutation and functional imaging results.

Tumor Suppressor Protein-53 (TP53)

Nineteen of twenty (95%) tumor tissue samples showed positive immunostaining for p53. Inter-reader correlation was high ($\sigma = 0.83$, $p < 0.01$). Representative images are shown in Figure 7. Primary tumor samples from patients with metastatic disease had greater cytoplasmic expression than tissue samples from patients without metastatic disease ($p < 0.04$). Presence of *VHL* mutations in exon 3 trended towards higher cytoplasmic expression of PTEN compared with patients with mutations in exon 1 or 2 ($p = 0.09$).

Discussion

In this study, we performed a comprehensive analysis of the protein expression of 8 genes by immunohistochemistry in VHL-associated PNETs to determine if any of these genes may be associated with clinical characteristics and outcome, imaging and genetic data. We found differences in subcellular compartment expression of PTEN, ATRX, CHGA and CHGB in VHL-associated PNETs which were associated with more aggressive tumors.

PTEN is a tumor suppressor gene with a well-established role as a susceptibility gene for Cowden's syndrome which has more recently been implicated in a variety of malignancies, including PNET. Jiao and associates, (10) identified a mutation rate of 7% in the *PTEN* gene in sporadic PNETs. Perren and colleagues (10, 17) identified loss of heterozygosity in *PTEN* in one-third of sporadic PNETs. In some, but not all studies, the level of PTEN expression was associated with a worse prognosis and poor response to chemotherapy in patients with PNETs. (15, 18-20) Several investigators have observed that the function of PTEN is dependent on subcellular localization throughout the cell cycle. (21-23) In the nucleus, PTEN maintains chromosome stability (24) and downregulates cyclin D1 with cell arrest, and thereby prevents activation of MAPK. (23, 25) On the other hand, cytoplasmic PTEN inhibits Akt, serving a pro-apoptotic function. (23, 26) Altered trafficking of PTEN has been described in other studies, but none have been in the context of VHL syndrome. (17, 27) In one study, no association was observed based on the subcellular localization of PTEN and clinical outcome. (17) In contrast, Missiaglia and colleagues (27) found that a low cytoplasmic expression of *PTEN* is associated with a shorter disease free interval. In our study, we found that patients with VHL-associated PNETs with a higher tumor nuclear-to-cytoplasmic PTEN expression ratio were more likely to have advanced disease by the WHO histologic classification. Interplay of the *mTOR/PTEN* pathway and *VHL* has been suggested in a variety of cancers. (28-31) These studies thus support our findings regarding altered PTEN subcellular localization in aggressive PNETs and higher cytoplasmic expression in FDG avid PNETs.

There is growing data of the role of *ATRX* and *DAXX* in oncogenesis. For example, *ATRX* and *DAXX* have been shown to interact with promyelocytic leukemia nuclear bodies (PML-NBs) and play a role in chromatin remodeling during S-phase. (15, 32-37) Recently, Heaphy

and colleagues (38) suggested a mechanism by which a loss of either ATRX or DAXX protein expression may occur as a result of altered telomere length in sporadic PNETs. Other investigators have also shown that ATRX regulates DNA methylation,(39-41) and RNA-mediated DNA methylation.(42) In the current study, all tissue samples had strong nuclear staining for ATRX and DAXX. However, altered N/C ATRX staining was observed with increased ratios in more aggressive tumors. To our knowledge, cytoplasmic localization of ATRX has only been described once in the literature, identified by immunofluorescence studies, and isolated to metaphase and anaphase of mitotic cells.(43) Typically, ATRX is described to dissociate with DAXX after chromatin remodeling occurs and then re-locates to the cytoplasm where it interacts with FAS ligand.(36, 44) The presence of cytoplasmic staining in our study may be due to more aggressive tumors having higher number of cells in metaphase and anaphase.

Chromogranins are a family of glycoproteins found in secretory vesicles within neuroendocrine cells. Chromogranin A (CHGA) is commonly used as a biomarker for neuroendocrine tumors: tissue CHGA is helpful for confirming a diagnosis of neuroendocrine cell type, whereas serum CHGA levels have been shown to correlate to patient tumor burden and may be helpful for patient surveillance.(11, 45) Chromogranin B (CHGB) has not been widely studied and does not have a defined clinical application presently. Several immunohistochemistry studies have shown varied results, with high expression related to a better prognosis in sporadic medullary thyroid cancer, worse prognosis in prostate cancer, and mixed results in pheochromocytomas.(46-49) This variety may be accounted for by the unique post-translational processing of the chromogranin family in different neuroendocrine tissues creating a different expression profile of epitopes.(50) Chromogranin's post-translational processing may even vary within tissue, as demonstrated in different expression patterns of different pancreatic islet cell types within normal pancreatic tissue.(51)

Immunostaining within our cohort of patients demonstrated that higher tumor grade was associated with lower nuclear expression of CHGA ($p=0.03$), with no significant difference in levels of cytoplasmic expression. Nuclear expression of CHGA has only been reported in one prior publication. The significance of this difference in subcellular localization is unclear.(52) CHGB immunostaining showed less cytoplasmic glycoprotein expression in primary tumors of patients with metastatic disease than in tissue samples from patients without metastatic disease ($p=0.03$). This finding is consistent with CHGB's cytoplasmic role of calcium ion regulation, which affects both cellular proliferation and cell death.(53, 54)

The most frequently mutated gene in human tumors is *TP53*, which encodes the p53 protein.(14) Usually, p53 expression is low in both the nucleus and cytoplasm of cells.(55-59) However, if a cell's DNA is damaged, p53 expression is increased and functions as a stress-responsive transcription factor.(55, 56) Roe, et al.(60) studied the interaction of pVHL and p53, identifying that the α -domain of pVHL directly associates with p53 to strengthen p53's transcriptional activity in the presence of DNA damage and to activate the p53 apoptotic signaling pathway by nucleating an ATM kinase. In VHL-related renal cell carcinomas, the alteration of the p53 regulatory pathway has been associated with a phenotype of high resistance to radiation and chemotherapy.(61, 62) Point mutations of p53 could also be a potential prognostic variable but this was not analyzed in our study.

In our cohort, cytoplasmic staining was significantly higher ($p<0.05$) in tumor samples taken from patients with metastatic disease, suggesting that in these samples p53 is sequestered in the cytoplasm, preventing it from engaging in its DNA stabilizing function. Similar staining has been described in breast cancer and lung cancer.(63-65) Yachida, et al., (15) identified a

loss of nuclear staining, not cytoplasmic staining, in poorly-differentiated PNETs. This correlated to intragenic mutations in the *TP53* gene as identified by sequencing analysis. The immunostaining difference seen in our cohort may be attributed to the interaction between pVHL and p53.

An interesting finding in our analysis was that *VHL* mutations located in exon 3 were associated with higher nuclear expression of *PTEN*. Indeed, mutations in exon 3 of the *VHL* gene have been associated with aggressive PNETs.(66) In our series, microcystic serous cystadenomas and WHO grade I (well-differentiated, benign features) PNETs had the highest nuclear expression, while WHO grade III had the lowest nuclear expression.

Another interesting finding of our study was the association between cytoplasmic expression of CC-3 and serum values of CHGA ($\sigma=0.7190$, $p=0.02$). A similar relationship has been reported in prostate cancer cell lines in which serum CHGA expression created a dose and time dependent cell-growth inhibition as measured by the number of apoptotic cells and CC-3 expression.(67) This suggests that serum CHGA may have a naturally intended biologic negative feedback roll on cell proliferation.

There are several limitations in our analysis. One is the number of samples analyzed doesn't allow us to evaluate the 8 genes as possible markers of disease-free survival and overall survival but this represents a relatively large study for VHL-associated PNETs. Although, immunohistochemistry is semiquantitative and may not detect relatively small differences in the expression level of the 8 genes studied, we wanted to use a standard technique which could be used in biopsy samples or histologic evaluation of tumors. This could allow the application of the markers, once validated, associated with aggressive PNETs to stratify which patients have aggressive tumor and would warrant resection. However, it will be important to validate our findings in a larger study cohort to make sure the significant statistical associations observed are not due to a limited sample set for this rare tumor.

This study examined the subcellular compartmentalization of 8 proteins to identify whether any provided prognostic value. Our results suggest this is true for several markers. A lower *PTEN* nuclear-to-cytoplasmic ratio, a higher *ATRX* nuclear to cytoplasmic ratio, a decreased expression of nuclear *CHGA*, a lower cytoplasmic expression of *CHGB* and a greater cytoplasmic expression of *TP53* are associated with aggressive VHL-associated PNETs. Furthermore, as each of these protein levels is independent of Ki-67 expression, these markers may act as an adjunctive marker for tumor grading.

Acknowledgments

This research was supported by the Intramural Research Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health.

References

1. Kim JJ, Rini BI, Hansel DE. Von Hippel Lindau syndrome. *Adv Exp Med Biol.* 2010; 685:228–49. Epub 2010/08/07. PubMed PMID: 20687511. [PubMed: 20687511]
2. Shehata BM, Stockwell CA, Castellano-Sanchez AA, Setzer S, Schmotzer CL, Robinson H. Von Hippel-Lindau (VHL) disease: an update on the clinico-pathologic and genetic aspects. *Adv Anat Pathol.* 2008; 15(3):165–71. Epub 2008/04/25. doi: 10.1097/PAP.0b013e31816f852e 00125480-200805000-00004 [pii]. PubMed PMID: 18434768. [PubMed: 18434768]
3. Kloppel G, Perren A, Heitz PU. The gastroenteropancreatic neuroendocrine cell system and its tumors: the WHO classification. *Ann N Y Acad Sci.* 2004; 1014:13–27. Epub 2004/05/22. PubMed PMID: 15153416. [PubMed: 15153416]

4. Rindi G, Kloppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, et al. TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Arch.* 2006; 449(4):395–401. Epub 2006/09/13. doi: 10.1007/s00428-006-0250-1. PubMed PMID: 16967267; PubMed Central PMCID: PMC1888719. [PubMed: 16967267]
5. Kloppel G. Classification and pathology of gastroenteropancreatic neuroendocrine neoplasms. *Endocr Relat Cancer.* 2011; 18(Suppl 1):S1–S16. Epub 2011/10/26. doi: 18/S1/S1 [pii] 10.1530/ERC-11-0013. PubMed PMID: 22005112. [PubMed: 22005112]
6. Schmitt AM, Anlauf M, Rousson V, Schmid S, Kofler A, Riniker F, et al. WHO 2004 criteria and CK19 are reliable prognostic markers in pancreatic endocrine tumors. *Am J Surg Pathol.* 2007; 31(11):1677–82. Epub 2007/12/07. doi: 10.1097/PAS.0b013e31805f675d 00000478-200711000-00007 [pii]. PubMed PMID: 18059224. [PubMed: 18059224]
7. Fischer L, Kleeff J, Esposito I, Hinz U, Zimmermann A, Friess H, et al. Clinical outcome and long-term survival in 118 consecutive patients with neuroendocrine tumours of the pancreas. *Br J Surg.* 2008; 95(5):627–35. Epub 2008/02/29. doi: 10.1002/bjs.6051. PubMed PMID: 18306152. [PubMed: 18306152]
8. La Rosa S, Klersy C, Uccella S, Dainese L, Albarello L, Sonzogni A, et al. Improved histologic and clinicopathologic criteria for prognostic evaluation of pancreatic endocrine tumors. *Hum Pathol.* 2009; 40(1):30–40. Epub 2008/08/22. doi: S0046-8177(08)00270-0 [pii] 10.1016/j.humpath.2008.06.005. PubMed PMID: 18715612. [PubMed: 18715612]
9. Scarpa A, Mantovani W, Capelli P, Beghelli S, Boninsegna L, Bettini R, et al. Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients. *Mod Pathol.* 2010; 23(6):824–33. Epub 2010/03/23. doi: modpathol201058 [pii] 1038/modpathol.2010.58. PubMed PMID: 20305616. [PubMed: 20305616]
10. Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science.* 2011; 331(6021):1199–203. Epub 2011/01/22. doi: science.1200609 [pii] 10.1126/science.1200609. PubMed PMID: 21252315; PubMed Central PMCID: PMC3144496. [PubMed: 21252315]
11. Portela-Gomes GM, Grimelius L, Wilander E, Stridsberg M. Granins and granin-related peptides in neuroendocrine tumours. *Regul Pept.* 2010; 165(1):12–20. Epub 2010/03/10. doi: S0167-0115(10)00047-9 [pii] 10.1016/j.regpep.2010.02.011. PubMed PMID: 20211659. [PubMed: 20211659]
12. Tomita T. Cleaved caspase-3 immunocytochemical staining for pancreatic islets and pancreatic endocrine tumors: A potential marker for biological malignancy. *Islets.* 2010; 2(2):82–8. Epub 2010/11/26. doi: 10807 [pii] 10.4161/isl.2.2.10807. PubMed PMID: 21099299. [PubMed: 21099299]
13. Kajdaniuk D, Marek B, Foltyn W, Kos-Kudla B. Vascular endothelial growth factor (VEGF) - part 2: in endocrinology and oncology. *Endokrynol Pol.* 2011; 62(5):456–64. Epub 2011/11/10. PubMed PMID: 22069107. [PubMed: 22069107]
14. Soussi T, Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. *Nat Rev Cancer.* 2001; 1(3):233–40. Epub 2002/03/21. doi: 10.1038/35106009. PubMed PMID: 11902578. [PubMed: 11902578]
15. Yachida S, Vakiani E, White CM, Zhong Y, Saunders T, Morgan R, et al. Small cell and large cell neuroendocrine carcinomas of the pancreas are genetically similar and distinct from well-differentiated pancreatic neuroendocrine tumors. *Am J Surg Pathol.* 2012; 36(2):173–84. Epub 2012/01/19. doi: 10.1097/PAS.0b013e3182417d36. PubMed PMID: 22251937; PubMed Central PMCID: PMC3261427. [PubMed: 22251937]
16. Kebebew E, Peng M, Treseler PA, Clark OH, Duh QY, Ginzinger D, et al. Id1 gene expression is up-regulated in hyperplastic and neoplastic thyroid tissue and regulates growth and differentiation in thyroid cancer cells. *J Clin Endocrinol Metab.* 2004; 89(12):6105–11. Epub 2004/12/08. doi: 10.1210/jc.2004-1234. PubMed PMID: 15579766. [PubMed: 15579766]
17. Perren A, Komminoth P, Saremaslani P, Matter C, Feurer S, Lees JA, et al. Mutation and expression analyses reveal differential subcellular compartmentalization of PTEN in endocrine pancreatic tumors compared to normal islet cells. *Am J Pathol.* 2000; 157(4):1097–103. Epub 2000/10/06. doi: S0002-9440(10)64624-X [pii] 10.1016/S0002-9440(10)64624-X. PubMed PMID: 11021813; PubMed Central PMCID: PMC1850183. [PubMed: 11021813]

18. Krausch M, Raffel A, Anlauf M, Schott M, Willenberg H, Lehwald N, et al. Loss of PTEN expression in neuroendocrine pancreatic tumors. *Horm Metab Res.* 2011; 43(12):865–71. Epub 2011/11/23. doi: 10.1055/s-0031-1291333. PubMed PMID: 22105477. [PubMed: 22105477]
19. Wang L, Ignat A, Axiotis CA. Differential expression of the PTEN tumor suppressor protein in fetal and adult neuroendocrine tissues and tumors: progressive loss of PTEN expression in poorly differentiated neuroendocrine neoplasms. *Appl Immunohistochem Mol Morphol.* 2002; 10(2): 139–46. Epub 2002/06/08. PubMed PMID: 12051632. [PubMed: 12051632]
20. O'Toole D, Couvelard A, Rebours V, Zappa M, Hentic O, Hammel P, et al. Molecular markers associated with response to chemotherapy in gastro-entero-pancreatic neuroendocrine tumors. *Endocr Relat Cancer.* 2010; 17(4):847–56. Epub 2010/06/24. doi: 10.1677/ERC-09-0204. PubMed PMID: 20570957. [PubMed: 20570957]
21. Orloff MS, Eng C. Genetic and phenotypic heterogeneity in the PTEN hamartoma tumour syndrome. *Oncogene.* 2008; 27(41):5387–97. Epub 2008/09/17. doi: onc2008237 [pii] 10.1038/onc.2008.237. PubMed PMID: 18794875. [PubMed: 18794875]
22. Ginn-Pease ME, Eng C. Increased nuclear phosphatase and tensin homologue deleted on chromosome 10 is associated with G0-G1 in MCF-7 cells. *Cancer Res.* 2003; 63(2):282–6. Epub 2003/01/25. PubMed PMID: 12543774. [PubMed: 12543774]
23. Planchon SM, Waite KA, Eng C. The nuclear affairs of PTEN. *J Cell Sci.* 2008; 121(Pt 3):249–53. Epub 2008/01/25. doi: 121/3/249 [pii] 10.1242/jcs.022459. PubMed PMID: 18216329. [PubMed: 18216329]
24. Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, et al. Essential role for nuclear PTEN in maintaining chromosomal integrity. *Cell.* 2007; 128(1):157–70. Epub 2007/01/16. doi: S0092-8674(06)01553-4 [pii] 10.1016/j.cell.2006.11.042. PubMed PMID: 17218262. [PubMed: 17218262]
25. Weng LP, Brown JL, Eng C. PTEN coordinates G(1) arrest by down-regulating cyclin D1 via its protein phosphatase activity and up-regulating p27 via its lipid phosphatase activity in a breast cancer model. *Hum Mol Genet.* 2001; 10(6):599–604. Epub 2001/03/07. PubMed PMID: 11230179. [PubMed: 11230179]
26. Chung JH, Eng C. Nuclear-cytoplasmic partitioning of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) differentially regulates the cell cycle and apoptosis. *Cancer Res.* 2005; 65(18):8096–100. Epub 2005/09/17. doi: 65/18/8096 [pii] 10.1158/0008-5472.CAN-05-1888. PubMed PMID: 16166282. [PubMed: 16166282]
27. Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M, et al. Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. *J Clin Oncol.* 2010; 28(2): 245–55. Epub 2009/11/18. doi: 10.1200/JCO.2008.21.5988. PubMed PMID: 19917848. [PubMed: 19917848]
28. Kucejova B, Pena-Llopis S, Yamasaki T, Sivanand S, Tran TA, Alexander S, et al. Interplay between pVHL and mTORC1 pathways in clear-cell renal cell carcinoma. *Mol Cancer Res.* 2011; 9(9):1255–65. Epub 2011/07/30. doi: 10.1158/1541-7786.MCR-11-0302. PubMed PMID: 21798997; PubMed Central PMCID: PMC3234675. [PubMed: 21798997]
29. von Teichman A, Comperat E, Behnke S, Storz M, Moch H, Schraml P. VHL mutations and dysregulation of pVHL- and PTEN-controlled pathways in multilocular cystic renal cell carcinoma. *Mod Pathol.* 2011; 24(4):571–8. Epub 2010/12/15. doi: 10.1038/modpathol.2010.222. PubMed PMID: 21151099. [PubMed: 21151099]
30. Petrella BL, Brinckerhoff CE. PTEN suppression of YY1 induces HIF-2 activity in von-Hippel-Lindau-null renal-cell carcinoma. *Cancer Biol Ther.* 2009; 8(14):1389–401. Epub 2009/06/02. PubMed PMID: 19483472; PubMed Central PMCID: PMC2761525. [PubMed: 19483472]
31. Rosner M, Hanneder M, Siegel N, Valli A, Fuchs C, Hengstschrager M. The mTOR pathway and its role in human genetic diseases. *Mutat Res.* 2008; 659(3):284–92. Epub 2008/07/05. doi: 10.1016/j.mrrev.2008.06.001. PubMed PMID: 18598780. [PubMed: 18598780]
32. Ishov AM, Vladimirova OV, Maul GG. Heterochromatin and ND10 are cell-cycle regulated and phosphorylation-dependent alternate nuclear sites of the transcription repressor Daxx and SWI/SNF protein ATRX. *J Cell Sci.* 2004; 117(Pt 17):3807–20. Epub 2004/07/15. doi: 10.1242/jcs.01230 jcs.01230 [pii]. PubMed PMID: 15252119. [PubMed: 15252119]

33. Lallemand-Breitenbach V, de The H. PML nuclear bodies. *Cold Spring Harb Perspect. Biol.* 2010; 2(5):a000661. Epub 2010/05/11. doi: cshperspect.a000661 [pii] 10.1101/cshperspect.a000661. PubMed PMID: 20452955; PubMed Central PMCID: PMC2857171.
34. Salomoni P, Betts-Henderson J. The role of PML in the nervous system. *Mol Neurobiol.* 2011; 43(2):114–23. Epub 2010/12/17. doi: 10.1007/s12035-010-8156-y. PubMed PMID: 21161613. [PubMed: 21161613]
35. Xue Y, Gibbons R, Yan Z, Yang D, McDowell TL, Sechi S, et al. The ATRX syndrome protein forms a chromatin-remodeling complex with Daxx and localizes in promyelocytic leukemia nuclear bodies. *Proc Natl Acad Sci U S A.* 2003; 100(19):10635–40. Epub 2003/09/04. doi: 10.1073/pnas.1937626100 1937626100 [pii]. PubMed PMID: 12953102; PubMed Central PMCID: PMC196856. [PubMed: 12953102]
36. Salomoni P, Khelifi AF. Daxx: death or survival protein? *Trends Cell Biol.* 2006; 16(2):97–104. Epub 2006/01/13. doi: S0962-8924(05)00310-7 [pii] 10.1016/j.tcb.2005.12.002. PubMed PMID: 16406523. [PubMed: 16406523]
37. de Wilde RF, Edil BH, Hruban RH, Maitra A. Well-differentiated pancreatic neuroendocrine tumors: from genetics to therapy. *Nat Rev Gastroenterol Hepatol.* 2012; 9(4):199–208. Epub 2012/02/09. doi: nrgastro.2012.9 [pii] 10.1038/nrgastro.2012.9. PubMed PMID: 22310917. [PubMed: 22310917]
38. Heaphy CM, de Wilde RF, Jiao Y, Klein AP, Edil BH, Shi C, et al. Altered telomeres in tumors with ATRX and DAXX mutations. *Science.* 2011; 333(6041):425. Epub 2011/07/02. doi: science.1207313 [pii] 10.1126/science.1207313. PubMed PMID: 21719641; PubMed Central PMCID: PMC3174141. [PubMed: 21719641]
39. Kernohan KD, Jiang Y, Tremblay DC, Bonvissuto AC, Eubanks JH, Mann MR, et al. ATRX partners with cohesin and MeCP2 and contributes to developmental silencing of imprinted genes in the brain. *Dev Cell.* 2010; 18(2):191–202. Epub 2010/02/18. doi: S1534-5807(10)00016-X [pii] 10.1016/j.devcel.2009.12.017. PubMed PMID: 20159591. [PubMed: 20159591]
40. Iwase S, Xiang B, Ghosh S, Ren T, Lewis PW, Cochrane JC, et al. ATRX ADD domain links an atypical histone methylation recognition mechanism to human mental-retardation syndrome. *Nat Struct Mol Biol.* 2011; 18(7):769–76. Epub 2011/06/15. doi: nsmb.2062 [pii] 10.1038/nsmb.2062. PubMed PMID: 21666679; PubMed Central PMCID: PMC3130887. [PubMed: 21666679]
41. Meehan RR, Stancheva I. DNA methylation and control of gene expression in vertebrate development. *Essays Biochem.* 2001; 37:59–70. Epub 2002/01/05. PubMed PMID: 11758457. [PubMed: 11758457]
42. Matzke MA, Birchler JA. RNAi-mediated pathways in the nucleus. *Nat Rev Genet.* 2005; 6(1):24–35. Epub 2005/01/05. doi: nrg1500 [pii] 10.1038/nrg1500. PubMed PMID: 15630419. [PubMed: 15630419]
43. Berube NG, Smeenk CA, Picketts DJ. Cell cycle-dependent phosphorylation of the ATRX protein correlates with changes in nuclear matrix and chromatin association. *Hum Mol Genet.* 2000; 9(4):539–47. Epub 2000/03/04. doi: ddd062 [pii]. PubMed PMID: 10699177. [PubMed: 10699177]
44. Khelifi AF, D'Alcontres MS, Salomoni P. Daxx is required for stress-induced cell death and JNK activation. *Cell Death Differ.* 2005; 12(7):724–33. Epub 2005/04/30. doi: 4401559 [pii] 10.1038/sj.cdd.4401559. PubMed PMID: 15861194. [PubMed: 15861194]
45. Modlin IM, Gustafsson BI, Moss SF, Pavel M, Tsolakis AV, Kidd M. Chromogranin A--biological function and clinical utility in neuro endocrine tumor disease. *Ann Surg Oncol.* 2010; 17(9):2427–43. Epub 2010/03/11. doi: 10.1245/s10434-010-1006-3. PubMed PMID: 20217257. [PubMed: 20217257]
46. Scopsi L, Sampietro G, Boracchi P, Collini P. Argyrophilia and chromogranin A and B immunostaining in patients with sporadic medullary thyroid carcinoma. A critical appraisal of their prognostic utility. *J Pathol.* 1998; 184(4):414–9. Epub 1998/07/17. doi: 10.1002/(SICI)1096-9896(199804)184:4<414::AID-PATH1229>3.0.CO;2-U [pii] 10.1002/(SICI)1096-9896(199804)184:4<414::AID-PATH1229>3.0.CO;2-U. PubMed PMID: 9664908. [PubMed: 9664908]
47. Pruneri G, Galli S, Rossi RS, Roncalli M, Coggi G, Ferrari A, et al. Chromogranin A and B and secretogranin II in prostatic adenocarcinomas: neuroendocrine expression in patients untreated and treated with androgen deprivation therapy. *Prostate.* 1998; 34(2):113–20. Epub 1998/02/18. doi:

- 10.1002/(SICI)1097-0045(19980201)34:2<113::AID-PROS5>3.0.CO;2-L [pii]. PubMed PMID: 9465942. [PubMed: 9465942]
48. Schmid KW, Helpap B, Totsch M, Kirchmair R, Dockhorn-Dworniczak B, Bocker W, et al. Immunohistochemical localization of chromogranins A and B and secretogranin II in normal, hyperplastic and neoplastic prostate. *Histopathology*. 1994; 24(3):233–9. Epub 1994/03/01. PubMed PMID: 7515371. [PubMed: 7515371]
 49. Portela-Gomes GM, Stridsberg M, Grimelius L, Falkmer UG, Falkmer S. Expression of chromogranins A, B, and C (secretogranin II) in human adrenal medulla and in benign and malignant pheochromocytomas An immunohistochemical study with region-specific antibodies. *APMIS*. 2004; 112(10):663–73. Epub 2004/12/17. doi: APMam1121003 [pii] 10.1111/j.1600-0463.2004.t01-1-apm1121003.x. PubMed PMID: 15601318. [PubMed: 15601318]
 50. Portel-Gomes GM, Grimelius L, Johansson H, Wilander E, Stridsberg M. Chromogranin A in human neuroendocrine tumors: an immunohistochemical study with region-specific antibodies. *Am J Surg Pathol*. 2001; 25(10):1261–7. Epub 2001/11/02. PubMed PMID: 11688460. [PubMed: 11688460]
 51. Portela-Gomes GM, Stridsberg M. Selective processing of chromogranin A in the different islet cells in human pancreas. *J Histochem Cytochem*. 2001; 49(4):483–90. Epub 2001/03/22. PubMed PMID: 11259451. [PubMed: 11259451]
 52. Montero-Hadjadje M, Vaingankar S, Elias S, Tostivint H, Mahata SK, Anouar Y. Chromogranins A and B and secretogranin II: evolutionary and functional aspects. *Acta Physiol (Oxf)*. 2008; 192(2):309–24. Epub 2007/11/17. doi: APS1806 [pii] 10.1111/j.1748-1716.2007.01806.x. PubMed PMID: 18005393. [PubMed: 18005393]
 53. Thrower EC, Choe CU, So SH, Jeon SH, Ehrlich BE, Yoo SH. A functional interaction between chromogranin B and the inositol 1,4,5-trisphosphate receptor/Ca²⁺ channel. *J Biol Chem*. 2003; 278(50):49699–706. Epub 2003/09/25. doi: 10.1074/jbc.M309307200 M309307200 [pii]. PubMed PMID: 14506248. [PubMed: 14506248]
 54. Capiod T, Shuba Y, Skryma R, Prevarskaya N. Calcium signalling and cancer cell growth. *Subcell Biochem*. 2007; 45:405–27. Epub 2008/01/16. PubMed PMID: 18193646. [PubMed: 18193646]
 55. Brooks CL, Gu W. Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. *Curr Opin Cell Biol*. 2003; 15(2):164–71. Epub 2003/03/22. doi: S0955067403000036 [pii]. PubMed PMID: 12648672. [PubMed: 12648672]
 56. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. *Nature*. 1997; 387(6630):299–303. Epub 1997/05/15. doi: 10.1038/387299a0. PubMed PMID: 9153396. [PubMed: 9153396]
 57. Shaulsky G, Goldfinger N, Ben-Ze'ev A, Rotter V. Nuclear accumulation of p53 protein is mediated by several nuclear localization signals and plays a role in tumorigenesis. *Mol Cell Biol*. 1990; 10(12):6565–77. Epub 1990/12/01. PubMed PMID: 2247074; PubMed Central PMCID: PMC362933. [PubMed: 2247074]
 58. Rotter V, Abutbul H, Ben-Ze'ev A. P53 transformation-related protein accumulates in the nucleus of transformed fibroblasts in association with the chromatin and is found in the cytoplasm of non-transformed fibroblasts. *EMBO J*. 1983; 2(7):1041–7. Epub 1983/01/01. PubMed PMID: 6354706; PubMed Central PMCID: PMC555232. [PubMed: 6354706]
 59. Gannon JV, Lane DP. Protein synthesis required to anchor a mutant p53 protein which is temperature-sensitive for nuclear transport. *Nature*. 1991; 349(6312):802–6. Epub 1991/02/28. doi: 10.1038/349802a0. PubMed PMID: 2000149. [PubMed: 2000149]
 60. Roe JS, Youn HD. The positive regulation of p53 by the tumor suppressor VHL. *Cell Cycle*. 2006; 5(18):2054–6. Epub 2006/09/14. doi: 3247 [pii]. PubMed PMID: 16969113. [PubMed: 16969113]
 61. Kawasaki T, Bilim V, Takahashi K, Tomita Y. Infrequent alteration of p53 pathway in metastatic renal cell carcinoma. *Oncol Rep*. 1999; 6(2):329–33. Epub 1999/02/19. PubMed PMID: 10022999. [PubMed: 10022999]
 62. Lowe SW. Cancer therapy and p53. *Curr Opin Oncol*. 1995; 7(6):547–53. Epub 1995/11/01. PubMed PMID: 8547404. [PubMed: 8547404]

63. Midgley CA, Fisher CJ, Bartek J, Vojtesek B, Lane D, Barnes DM. Analysis of p53 expression in human tumours: an antibody raised against human p53 expressed in *Escherichia coli*. *J Cell Sci*. 1992; 101(Pt 1):183–9. Epub 1992/01/01. PubMed PMID: 1569122. [PubMed: 1569122]
64. Moll UM, Riou G, Levine AJ. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. *Proc Natl Acad Sci U S A*. 1992; 89(15):7262–6. Epub 1992/08/01. PubMed PMID: 1353891; PubMed Central PMCID: PMC49686. [PubMed: 1353891]
65. Iggo R, Gatter K, Bartek J, Lane D, Harris AL. Increased expression of mutant forms of p53 oncogene in primary lung cancer. *Lancet*. 1990; 335(8691):675–9. Epub 1990/03/24. doi: 0140-6736(90)90801-B [pii]. PubMed PMID: 1969059. [PubMed: 1969059]
66. Blansfield JA, Choyke L, Morita SY, Choyke PL, Pingpank JF, Alexander HR, et al. Clinical, genetic and radiographic analysis of 108 patients with von Hippel-Lindau disease (VHL) manifested by pancreatic neuroendocrine neoplasms (PNETs). *Surgery*. 2007; 142(6):814–8. discussion 8 e1-2. Epub 2007/12/08. doi: S0039-6060(07)00529-6 [pii] 10.1016/j.surg.2007.09.012. PubMed PMID: 18063061. [PubMed: 18063061]
67. Yu DS, Hsieh DS, Chang SY. Modulation of prostate carcinoma cell growth and apoptosis by chromogranin A. *J Urol*. 2003; 170(5):2031–5. Epub 2003/10/09. doi: 10.1097/01.ju.0000091807.02246.f3 S0022-5347(05)63014-5 [pii]. PubMed PMID: 14532847. [PubMed: 14532847]

Figure 1.

Representative PTEN expression by immunohistochemistry. A: Normal islet cells (magnified 20x), B: Microcystic Serous Cystadenoma (magnified 20x), C: Well differentiated neuroendocrine tumor, benign characteristics (magnified 20x), D: Well differentiated neuroendocrine tumor, undetermined characteristics (magnified 20x), E: Neuroendocrine carcinoma (magnified 20x), Bottom Right: Immunohistochemistry scoring averages by diagnosis.

Figure 2. Level of PTEN nuclear expression by *VHL* mutation location. Box plot represents PTEN nuclear score. PTEN expression by immunohistochemistry was significantly different ($p=0.04$) with only an 18% overlap in PTEN expression level by exon mutation status.

Figure 3. PTEN cytoplasmic expression by ^{18}F -FDG-PET avidity. Box plot represents the TEN cytoplasmic expression by the presence or absence of an ^{18}F -FDG PET avid tumor on imaging. PTEN expression was significantly different ($p=0.04$) by ^{18}F -FDG PET avidity status.

Figure 4.

ATRX expression by immunohistochemistry in representative tissue samples. A: Normal islet cells (magnified 20x), B: Microcystic Serous Cystadenoma (magnified 20x), C: Well differentiated neuroendocrine tumor, benign characteristics (magnified 20x), D: Well differentiated neuroendocrine tumor, undetermined characteristics (magnified 20x), E: Neuroendocrine carcinoma (magnified 20x), Bottom Right: ATRX expression level by diagnosis.

Figure 5.

CHGA expression by immunohistochemistry in representative tissue samples. A: Normal islet cells (magnified 20x), B: Microcystic Serous Cystadenoma (magnified 20x), C: Well differentiated neuroendocrine tumor, benign characteristics (magnified 20x), D: Well differentiated neuroendocrine tumor, undetermined characteristics (magnified 20x), E: Neuroendocrine carcinoma (magnified 20x), Bottom Right: CHGA expression level by diagnosis.

Figure 6.

CHGB expression by immunohistochemistry in representative tissue samples. A: Negative Control (magnified 20x), B: Normal Islet Cells, in patient with VHL (magnified 20x), C: Non-metastatic tumor (magnified 20x), D: Metastatic tumor (magnified 20x), Bottom Right: CHGB expression level by diagnosis.

Table 1

Clinical characteristics of the study cohort

Clinical Feature	Number
Number of Patients	21
Sex	
Male	8
Female	13
Age, years	48.5 ± 13.4
Range	19 to 70
Pathology Diagnosis	
Normal Islet Cells	4
Microcystic Serous Cystadenoma	4
PNET, Well-differentiated, Benign	4
PNET, Well-differentiated, Uncertain	7
PNET, Neuroendocrine Carcinoma	4
FDG-PET Avid	
Yes	10
No	11
Serum Chromogranin A (reference normal < 93 ng/ml)	
Mean	212
Range	98 - 1,105