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CASE REPORT

# Inhibin-expressing clear cell neuroendocrine tumor of the ampulla: an unusual presentation of von Hippel–Lindau disease

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**Abstract** von Hippel–Lindau (VHL) disease is a hereditary autosomal dominant disorder associated with deletions or mutations in the VHL tumor suppressor gene. Characteristically, up to 60 % of neuroendocrine tumors (NETs) associated with VHL disease display a spectrum of clear cell morphology including multivacuolated lipid-rich cell change. Unlike neurofibromatosis type 1 and multiple endocrine neoplasia type 1 syndromes, ampullary NETs have not been described in association with VHL disease. In this report, we discuss the features of an incidental ampullary clear cell NET occurring in a patient with pancreatic VHL disease including multiple pancreatic NETs. The ampullary lesion consisted of epithelial cells resembling lipoblasts or signet ring cells. In our case, all NETs showing clear cell change were positive for inhibin. While the underlying mechanism of this finding is largely unknown, it is of note that positivity for inhibin has not been observed in clear cell NETs associated with multiple endocrine neoplasia type 1 syndrome. Our case proves that NETs can develop in the ampullary region in patients with VHL; clear cell change can occur in these lesions and can mimic signet ring cell carcinoma. This issue is of clinical significance especially in small biopsy samples; thus, positivity for keratin alone should not be taken as evidence of an adenocarcinoma. Moreover, demonstration of inhibin expression in a NET with clear cell change along with other clinical stigmata should alert the diagnostician to the possibility of VHL disease. However, further larger series examining inhibin expression in both syndrome-related and sporadic clear cell NETs are needed to confirm our findings.

**Keywords** von Hippel–Lindau · Inhibin · Clear cell neuroendocrine tumor · HIF · Pancreas · Ampulla

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## Introduction

von Hippel–Lindau (VHL) disease is a hereditary autosomal dominant disorder associated with deletions or mutations in the VHL tumor suppressor gene [1, 2]. The disease is typically characterized by retinal angiomas; central nervous system hemangioblastomas; hemangiomas of the adrenal, liver, and lung; cysts in the kidney, pancreas, epididymis, and liver; as well as endolymphatic sac tumors, renal cell carcinoma, and neuroendocrine tumors (NETs) including pheochromocytomas and paragangliomas as well as pancreatic NETs [1–3]. Characteristically, up to 60 % of NETs associated with VHL disease display a spectrum of clear cell morphology including multivacuolated lipid-rich cell change [2, 3]. Unlike neurofibromatosis type 1 (NF-1) and multiple endocrine neoplasia type 1 (MEN1) syndromes, ampullary NETs have not been described in association with VHL disease [2–5]. In this report, we discuss the features of an incidental ampullary clear cell NET occurring in a patient with pancreatic VHL disease.

## Clinical history

A 60-year-old woman with known history of VHL disease was found to have multiple pancreatic cysts as well as two octreotide-avid pancreatic lesions, consistent with NETs on

magnetic resonance and OctreoScan imaging studies. Her past medical history revealed that she had undergone previous left adrenalectomy for a pheochromocytoma and a partial pancreatectomy and splenectomy for a cystic pancreatic lesion. She had a cerebral hemangioblastoma for which she had undergone angioembolization. Recently, she was also found to have a small left renal mass which was being monitored by surveillance. Molecular analysis of germline DNA confirmed a disease-causing mutation in the VHL gene (c.4641G>A).

## Materials and methods

Given the growth rate and multifocal nature of her pancreatic NETs, a completion pancreatectomy/Whipple resection was performed (Fig. 1a). The specimen was fixed in 10 % buffered formalin. Sections were embedded in paraffin and 3-μm sections were cut for hematoxylin and eosin staining and for immunohistochemical analysis.

## Results

The resected pancreas contained numerous serous microcystic adenomas along with multiple grade 2 NETs (WHO 2010) with variable clear cell change (Figs. 1a, b and 2a, b). The dominant 4.0-cm tumor exhibited angioinvasion, characterized by intravascular tumor cells with adherent thrombus. The other tumors measured 0.9 and 0.7 cm. The MIB-1 labeling index was 6, 3, and 3 % in the 4.0-, 0.9-, and 0.7-cm tumors, respectively. The mitotic activity was less than 2/10 high-power fields in all tumors. Two smaller endocrine microadenomas measured 0.4 and 0.25 cm. All tumors were confined to the pancreas and the surgical margins were negative for neoplastic infiltration. Thirty-four lymph nodes were dissected from the specimen and all were negative for metastatic disease. All tumors were positive for CAM5.2, AE1/AE3, chromogranin A, and synaptophysin. All five clear cell pancreatic NETs were positive for pancreatic polypeptide and inhibin (Fig. 2c, d); the 4.0-cm dominant tumor also expressed somatostatin and peptide-YY. There was no evidence of peliosis, nesidioblastosis, or nesidiodysplasia. The overall morphological features were consistent with the morphological spectrum of VHL disease.

Interestingly, an incidental tubular adenoma with low-grade dysplasia was found in the ampulla (Fig. 3a). Adjacent to this lesion, there was a 0.2-cm epithelial neoplasm in the duodenal ampullary mucosa (Fig. 3a–c). This lesion consisted of epithelial cells resembling lipoblasts or signet ring cells. No mitotic figures were noted. Keratins (cytokeratin 7, AE1/AE3, and CAM5.2; Fig. 3d), synaptophysin, and chromogranin A (Fig. 3e) were strongly expressed in this lesion, consistent with a NET. This lesion was negative for cytokeratin 20 and mucin stains and showed inhibin immunoreactivity (Fig. 3f). The MIB-1 labeling index was 3 %. Preoperative biochemical hormone levels were not available; however, postoperative laboratory testing showed an increased level of pancreatic polypeptide, suggestive of residual microscopic disease.

## Discussion

NETs comprise approximately 2 % of ampullary neoplasms [5]. They have been described in the setting of NF-1 and MEN1 syndromes or as sporadic lesions [2–5]. While VHL disease has been associated with gallbladder, extrahepatic bile duct, or pancreatic NETs with clear cell change [2, 3, 5–8], ampullary NETs have not been described in this syndrome.

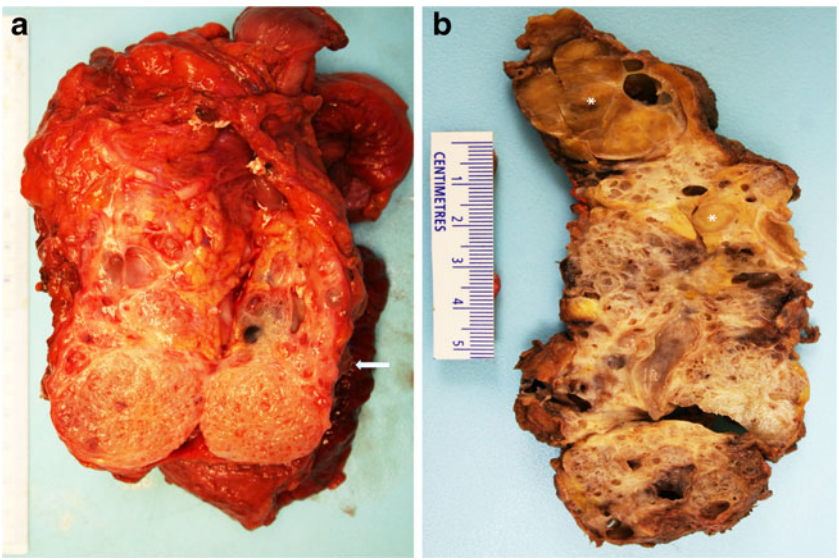


**Fig. 1** Pancreatic gross pathology in VHL. **a** The completion pancreatectomy/Whipple specimen contains multiple solid and cystic lesions in the pancreas and a small lesion in the ampullary mucosa (*arrow*). **b** After fixation in formalin, the neuroendocrine tumors exhibit a conspicuous yellow appearance (*asterisk*) and are readily distinguished from the microcystic pancreatic parenchyma

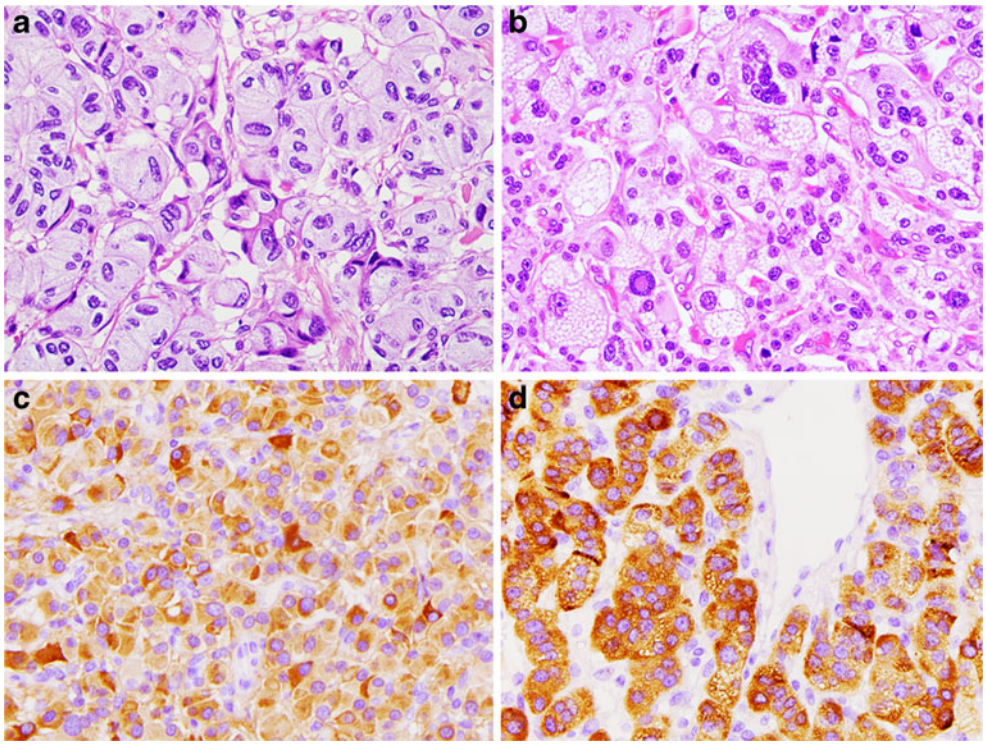


**Fig. 2** Pancreatic endocrine pathology in VHL. The various NETs showed variable clear cell change: the dominant tumor exhibits glassy clear cell change (**a**) and the smaller tumor has a vacuolated lipid pattern (**b**). These tumors were both positive for inhibin (**c**, **d**)

The pancreatic involvement of VHL disease is typically characterized by benign cysts and microcystic or serous adenomas [2–4, 9]. MEN1 pancreata have been long known to exhibit multifocal NETs including pancreatic endocrine microadenomas (less than 0.5 cm), as well as nesidioblastosis (ductuloinsular complexes), nesidiodysplasia, and peliosis; however, similar findings have also been identified in pancreatic VHL disease [2, 3, 8, 10].

Clear cell change in NETs including paragangliomas and pheochromocytomas is usually thought to be a characteristic feature of VHL disease, especially in the context of multifocal pancreatic disease [2, 3, 6]. It can be quite dramatic, creating a diagnostic dilemma [11]. This phenomenon is often attributed to the activation of hypoxia-inducible factor 1 alpha (HIF-1alpha), leading to a status of pseudohypoxia resulting in lipid and glycogen accumulation in tumor cells [1, 12–15]. It has been shown that HIF-mediated stimulation of lipin 1 expression causes triglyceride accumulation [13]. Moreover, HIF-1alpha promotes LDL and VLDL uptake through regulation of VLDLR gene expression [14]. Glycogen synthesis is induced in hypoxia by HIF-1 and promotes cancer cell survival [15]. Recent evidence also suggests that clear cell change can also be seen rarely in patients with MEN1 syndrome as well as in sporadic tumors due to cell senescence [16–21] (Table 1).

Interestingly, inhibin positivity has been reported in clear cell NETs associated with VHL syndrome [6]. In our case, all NETs showing clear cell change were positive for inhibin. While the underlying mechanism of this finding is largely unknown, this phenomenon may be attributable to the status of pseudohypoxia resulting in HIF-1alpha increase in the setting of VHL disease. Evidence suggests that an increase in HIF-1alpha leads to the induction of VEGF, inhibin, and LHR [22]. Of note, inhibin and activin play an important role in the regulation of the steroidogenesis and inhibin blocks the suppressive effective of activin [23]. It has been shown that activin inhibits adipogenesis via affecting lipid accumulation, expression of glycerol-3-phosphate dehydrogenase activity, and expression of adipocyte fatty acid-binding protein mRNA [24]. Thus, the positivity for inhibin may be attributable to lipid accumulation modulated via HIF-1alpha. It is of note that positivity for inhibin has exclusively reported in patients with clear cell NETs associated with VHL disease and in one sporadic clear cell NET (Table 1). However, positivity for inhibin has not been observed in other clear cell NETs associated with MEN1 syndrome [16]. Thus, inhibin immunohistochemistry may be used to suggest the possibility of VHL disease underlying multifocal clear cell NETs. However, this issue should be further clarified in larger series that also include sporadic NETs with clear cell change.

Our case clearly indicates that NETs can develop in the ampullary region in patients with VHL; clear cell change can occur in these lesions and can mimic signet ring cell carcinoma. This issue is of clinical significance especially in small biopsy samples; thus, positivity for keratin alone should not be taken as evidence of an adenocarcinoma. Moreover, demonstration of

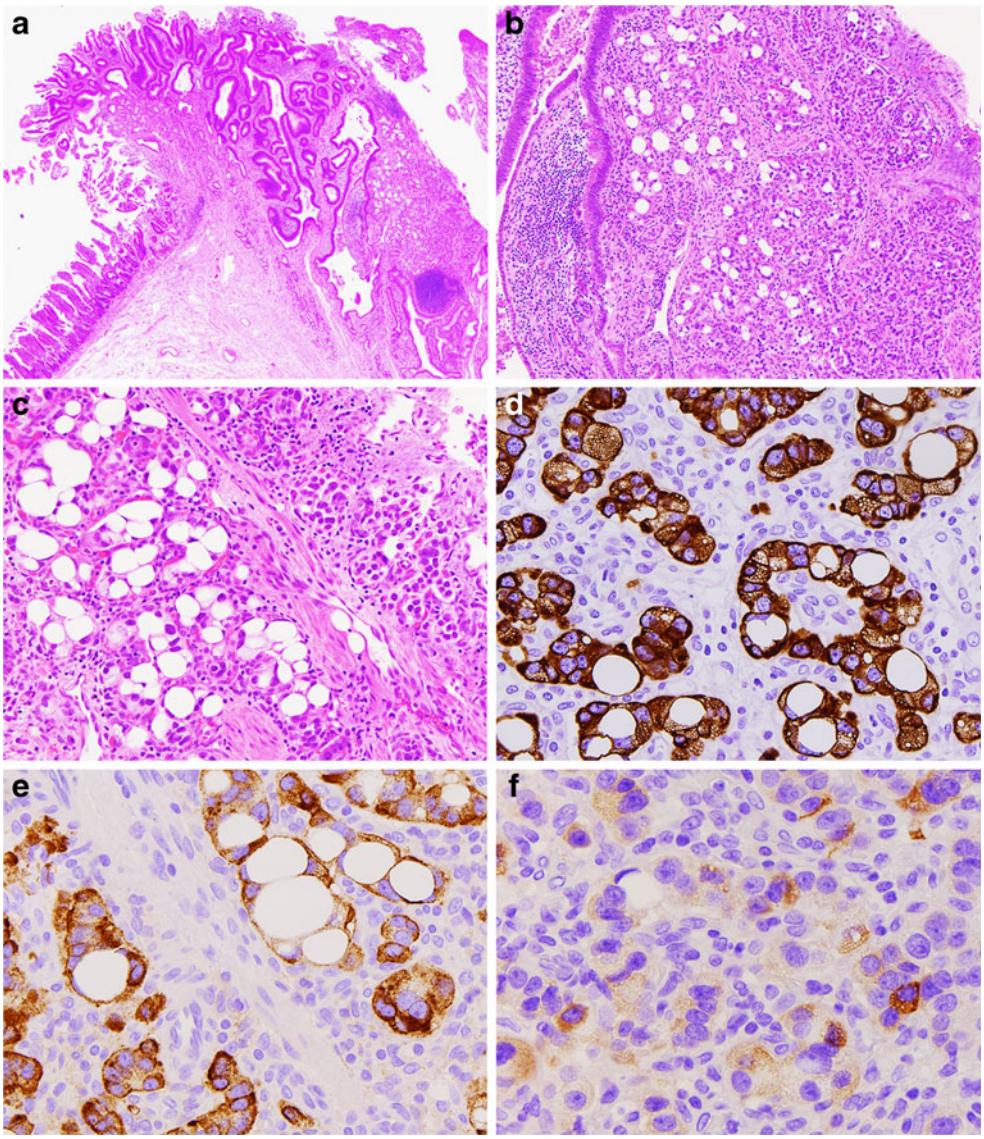


**Fig. 3** Ampullary pathology in VHL. In addition to pancreatic VHL disease, an incidental tubular adenoma with low-grade dysplasia was noted in the ampulla (**a**). Adjacent to this lesion, there was a 0.2-cm neuroendocrine neoplasm in the ampulla (**a**–**c**). This lesion consisted of epithelial cells resembling lipoblasts or signet ring cells (**a**–**c**). These cells were diffusely positive for CAM5.2 (**d**) and chromogranin A (**e**) and were variably positive for inhibin (**f**)

**Table 1** Clear cell neuroendocrine tumors with respect to their inhibin expression

| Study | Number of clear cell NETs reported | Anatomic localization | Sporadic vs familial | Inhibin positivity |
|---|---|---|---|---|
| Todoroki et al. [17] | 1 | Common bile duct | Sporadic | Absent |
| Konishi et al. [18] | 1 | Gallbladder | Sporadic | Absent |
| Sinkre et al. [6] | 5 | Gallbladder (*n*=1), pancreas (*n*=4) | VHL (*n*=5) | All tumors were positive |
| Ishida et al. [19] | 1 | Gallbladder | Sporadic | Absent |
| Fryer et al. [16] | 6 | Pancreas | MEN1 | Absent |
| Chetty et al. [20] | 7 | Appendix vermiformis | Sporadic | Absent |
| Singh et al. [21] | 11 | Pancreas | VHL (*n*=1), MEN1 (*n*=1), sporadic (*n*=9) | One sporadic case was positive |
| Current case | 1 | Ampulla and pancreas | VHL | All tumors are positive |

*n* number of cases with respect to their association with sporadic or familial disease. NETs stand for neuroendocrine tumors

inhibin expression in a NET with clear cell change (especially in the setting of multifocal disease) along with other clinical stigmata should alert the diagnostician to the possibility of VHL disease.

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