

Targeted Sequencing of Mixed Neuroendocrine–Non-neuroendocrine Neoplasm of the Gallbladder Suggests a Monoclonal Origin: A Case Report

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ABSTRACT

Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) of the gallbladder is a rare tumor that is defined in the World Health Organization (WHO) 2019 digestive system tumor classification as the presence of a neuroendocrine neoplasm admixed with a non-neuroendocrine carcinoma, each component constituting at least 30% of the neoplasm. The exact pathogenesis of MiNENs remains unclear. We present a case of a 74-year-old Filipino woman who presented with nonspecific clinical and radiologic findings and subsequently underwent cholecystectomy. Histopathologic and immunohistochemical evaluation of the gallbladder confirmed the diagnosis of a mixed well-differentiated adenocarcinoma (30%) and large cell neuroendocrine carcinoma (70%). The adenocarcinoma and neuroendocrine carcinoma components were separately microdissected and submitted for targeted 15-gene sequencing using the Illumina TruSight Tumor 15 (TST15) panel. NGS identified a TP53 missense mutation leading to a stop codon in both components. The finding of similar molecular signatures in the two morphologically distinct components supports the hypothesis that MiNEN arises from a common precursor stem cell capable of divergent phenotypic differentiation.

Key words: gallbladder, MiNEN, molecular analysis

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INTRODUCTION

Gallbladder neuroendocrine neoplasms (NENs) account for 0.5% of all NENs and 2.1% of gallbladder cancers.¹⁻³ Of the different types of gallbladder NENs, large cell neuroendocrine carcinoma (LCNEC) is an aggressive and exceptionally rare tumor, with about 20 cases of both pure and mixed types reported since 2000.^{1,4-7} Primary mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) of the gallbladder is even more rare. MiNENs are characterized by the neuroendocrine tumors or carcinomas with a non-neuroendocrine carcinoma component, each carcinoma type constituting at least 30% of the tumor. MiNENs were traditionally classified by Lewin into collision, composite and amphicrine types, on the basis of the association between, and the immunophenotype of the two components.^{4,7,8} We herein report a case of mixed adenocarcinoma and LCNEC of the gallbladder with immunohistochemical evaluation and molecular testing.

CASE

A 74-year-old Filipino woman with no known comorbidities experienced right upper abdominal pain and fever of three-day duration. Persistence of the abdominal pain prompted her to seek consult in the emergency department. Physical examination revealed direct tenderness in the right upper quadrant, a positive Murphy's sign and mild icterus. Blood tests were unremarkable and non-contributory. Initial impression was acute calculous cholecystitis. Endoscopic retrograde cholangiopancreatography (ERCP) revealed stenotic ampulla of Vater and an obstructed cystic duct.



Whole abdominal CT scan (Figures 1A and B) demonstrated a distended multiseptated gallbladder (maximum width of 4.3 cm) with a slightly hyperdense lumen (suggestive of bile sludge) and a 1.1 cm non-enhancing faintly hyperdense ovoid focus (suggest a low-density cholelithiasis versus sludge ball) within the body. Mild thickening of the gallbladder wall with extensive pericholecystic fat stranding (reflective of cholecystitis), and a focal defect along the left anteromedial portion of the gallbladder were also seen. There was moderate dilatation of the cystic duct, intra- and extrahepatic ducts and common bile duct, without discrete signs of calcified choledocholithiasis. The gastric pylorus, first and second portion of the duodenum, hepatic flexure/ascending colon all showed wall thickening/edema, likely reactive, and the pancreas is atrophic. There were no other significant pathologic lesions in the imaging studies.

Open cholecystectomy was then performed and the gallbladder was sent for routine histopathologic examination. Gross examination revealed the gallbladder (Figure 1C) to have a tan brown, dull and rough external surfaces, with multiple fine adhesions and thickened wall (1 cm.). The gallbladder lumen is completely filled with a cream tan, variegated, polypoid, soft to friable mass (6.1 cm in greatest dimension) that is loosely attached to the luminal surface, which had brown tan and flattened mucosa. No yellow flecks nor stones appreciated. No lymph nodes submitted for gross examination. Microsections disclosed a neoplasm with two distinct morphologies (Figures 2A-2C; Figure 3): a poorly differentiated carcinoma and a well differentiated adenocarcinoma, that are closely juxtaposed but with apparent transition. The poorly differentiated carcinoma (Figure 4A), which comprised the majority of the tumor, is composed of large, round to pleomorphic cells with vesicular nuclei, prominent nucleoli and scant to moderate amount of cytoplasm. These cells, arranged in solid sheets, palisades and rosette-like and pseudoglandular patterns, invaded up to the perimuscular connective tissue of the organ without serosal involvement. This component is associated with abundant mitotic figures (83/50 high power fields) with rare atypical mitoses, extensive geographic necrosis, and demonstrated a high (95%) Ki67 proliferation index (Figure 4F). The adenocarcinoma component, which constitute 30% of the tumor, is composed of atypical columnar epithelial cells with enlarged, hyperchromatic nuclei. The cells exhibit stratification and crowding and display glandular, cribriform and papillary formations. Lymphovascular and perineural invasion were seen, and the cystic duct margin was uninvolved by the tumor. The non-neoplastic mucosa showed intestinal metaplasia and chronic inflammatory infiltrates (Figure 2D).

Immunohistochemical studies

Immunohistochemical staining was performed as previously described (Roche BenchMark ULTRA and Leica BOND-MAX IHC/ISH systems) on a representative section to better characterize the poorly differentiated carcinoma component of the tumor. Immunoreactivity (Figures 4B-4E) of the poorly differentiated component to neuroendocrine markers [chromogranin A (LK2H10) and synaptophysin (SP11)], cytokeratin (AE1/AE3/PCK26) and CK19 (b170) confirmed the diagnosis of a large cell neuroendocrine carcinoma. Hence, a final diagnosis of

mixed well-differentiated adenocarcinoma and poorly differentiated (large cell) neuroendocrine carcinoma was reported. As per the staging of gallbladder carcinoma, the patient was stage IIA.

Molecular studies

The non-neuroendocrine (adenocarcinoma) and neuroendocrine carcinoma components were carefully microdissected from 5 μ m thick paraffin-embedded tissue slices. DNA from each component was extracted using the QIAamp DNA FFPE tissue kit (Qiagen), according to the manufacturer's instructions. Both components were individually submitted for next-generation sequencing (NGS) using the Illumina (San Diego, CA) Trusight Tumor 15 (TST15) kit and subsequently sequenced using Illumina MiSeq® system. Along with all the exons of TP53, selected regions of the following genes were sequenced: AKT1, BRAF, EGFR, ERBB2, FOXL2, GNA11, GNAQ, KIT, KRAS, MET, NRAS, PDGFRA, PIK3CA and RET. Mutational analysis identified a TP53 missense mutation (Figure 3) that leads to a stop codon (c.273G>A, p.Trp91Ter) in both components.

Follow-up

Patient was lost to follow up and was asymptomatic until seven months later she decided to consult her surgeon due to a palpable abdominal mass. She is scheduled to undergo another whole abdominal CT scan.

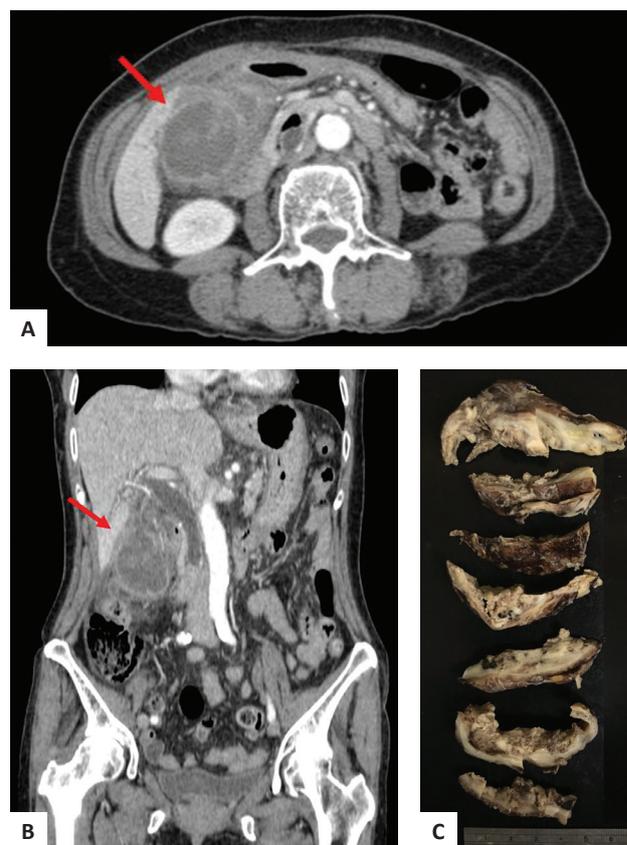


Figure 1. Whole abdominal CT scan, (A) axial and (B) coronal slices. The gallbladder (red arrow) is distended with hyperdense lumen suggestive of bile sludge. (C) The gallbladder wall is thickened with extensive pericholecystic fat stranding reflective of cholecystitis. The gallbladder has tan brown external surfaces with fine adhesions and thickened wall.

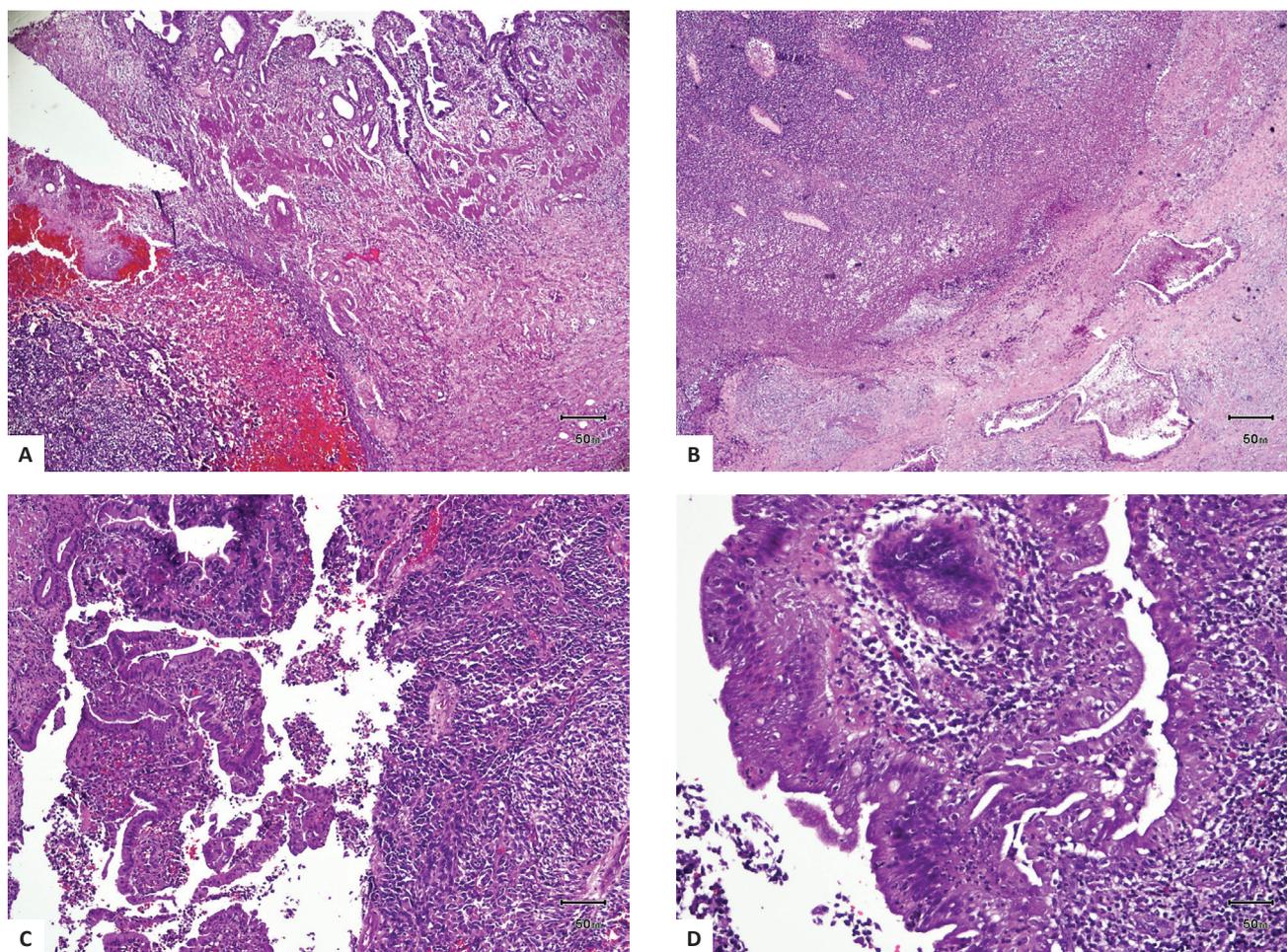


Figure 2. Hematoxylin & eosin stain [H&E]. (A and B) [H&E, 4X] and (C) [H&E, 10X] MiNEN showing juxtaponition of the well-differentiated adenocarcinoma and the poorly differentiated carcinoma components. (D) Intestinal metaplasia, as evidenced by the presence of scattered goblet cells in the mucosa, and chronic inflammatory infiltrates were seen in the non-neoplastic portion of the gallbladder [H&E, 20X].

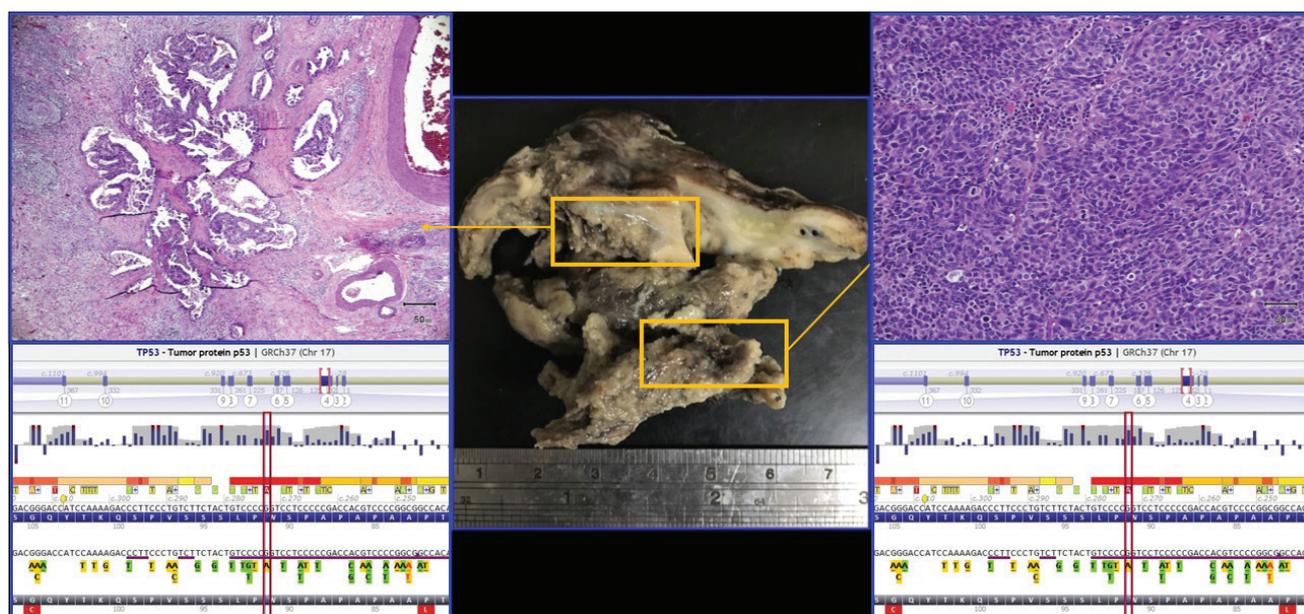


Figure 3. The adenocarcinoma component on the left exhibits cribriform, papillary and glandular patterns. The neuroendocrine component on the right is composed of solid sheets of large cells with hyperchromatic nuclei. Both components are intimately admixed with chronic inflammatory cells. Next generation sequencing revealed an identical TP53 missense mutation that leads to a stop codon (c.273G>A, p.Trp91Ter) in the two components [H&E, 4X].

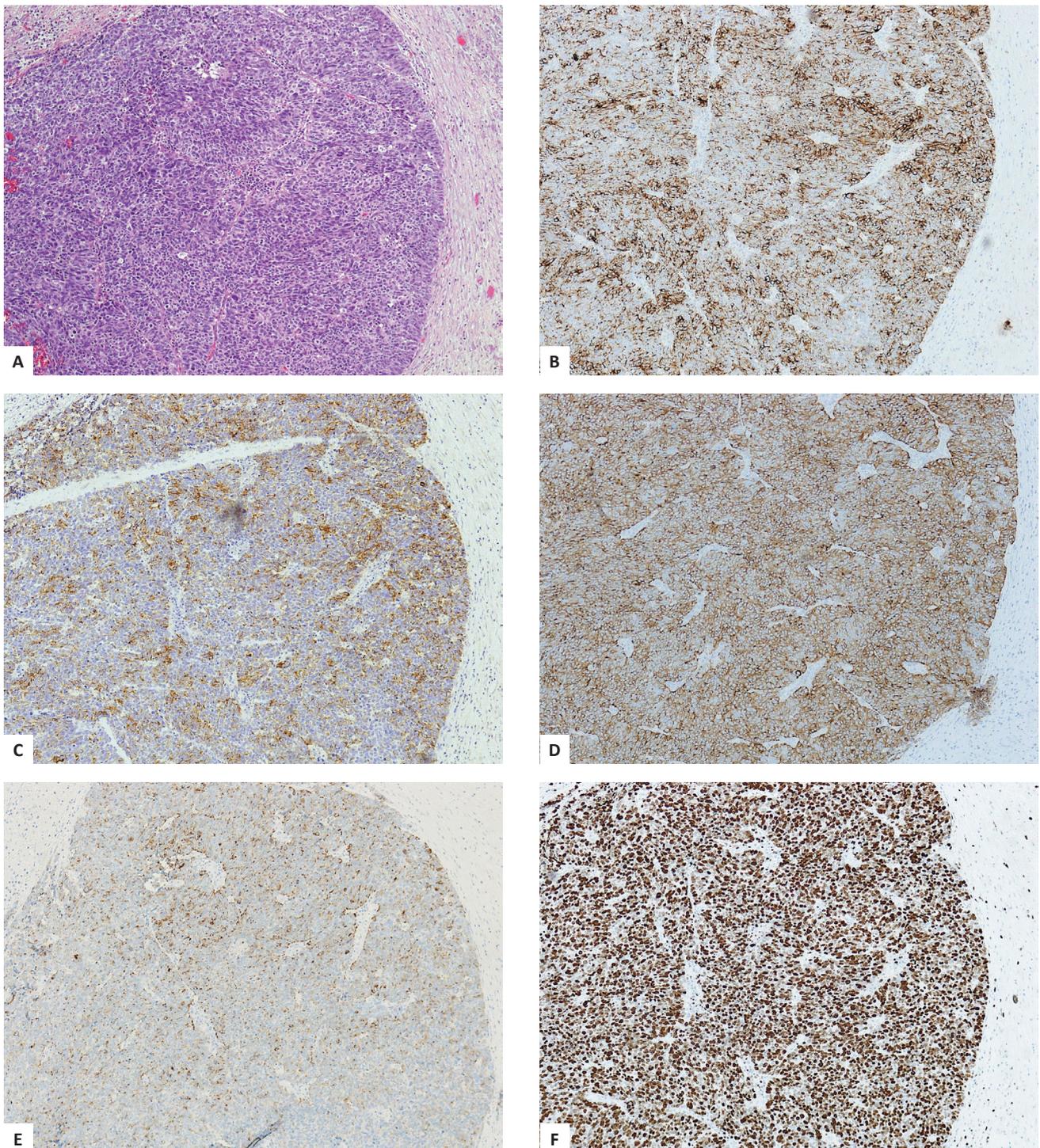


Figure 4. Hematoxylin & eosin stain [H&E]. **(A)** The poorly differentiated neuroendocrine component shows large, round to pleomorphic cells with vesicular nuclei, prominent nucleoli and scant to moderate amount of cytoplasm [H&E, 10X]. These cells are arranged in trabeculae and pseudoglandular formations. This carcinoma component displays immunoreactivity to CK [AE1/AE3, 10X] **(B)**, CK19 [10X] **(C)**, Synaptophysin [10X] **(D)**, and Chromogranin [10X] **(E)** A high (95%) Ki67 [10X] **(F)** Proliferation index is appreciated.

DISCUSSION

Neuroendocrine neoplasms of the gallbladder and bile ducts were subtyped in the WHO 2019 tumor classification based on the mitotic activity and Ki67 proliferation index. The categories include neuroendocrine tumor (NET) grade 1, NET grade 2, NET grade 3, large cell neuroendocrine carcinoma, small cell neuroendocrine carcinoma. Poorly differentiated neuroendocrine carcinoma (PDNEC) which include small cell and large cell neuroendocrine carcinoma (LCNEC), are characterized by brisk mitotic activity (>20 mitoses/ 10 HPFs) and Ki67 proliferation index of more than 20%, with or without necrosis. These neoplasms can occur in the pure form or may be admixed with other histologic components as in cases of mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN). Grossly, LCNEC of the gallbladder NENs appear as infiltrative polypoid, nodular or cauliflower-shaped masses with homogeneous cut surfaces, that invade the muscular wall, with or without extension to the serosa. On microscopic examination, LCNEC is composed of large polygonal cells about three times the lymphocyte diameter. These pleomorphic cells have low nuclear to cytoplasmic ratio, vesicular nuclei, conspicuous nucleoli and abundant cytoplasm. They exhibit peripheral palisading and grow in trabeculae, cords, sheets, pseudoglandular or rosette-like patterns.^{1,2,4,9,10} Although at a reduced extent and intensity as compared with well-differentiated tumors, PDNECs are generally immunoreactive to neuroendocrine markers (Synaptophysin, Chromogranin and CD56), a criterion required for the diagnosis of LCNEC. At the molecular level, PDNECs of the gastrointestinal and pancreaticobiliary tracts show TP53 and retinoblastoma gene (RB1) mutations. LCNEC are aggressive tumors that metastasize early, and are associated with a poor prognosis.^{2,9,4}

Mixed neuroendocrine-non-neuroendocrine neoplasm, previously referred to as mixed adenocarcinoma and neuroendocrine carcinoma (MANEC) in the 2010 WHO blue book, is defined as a tumor histologically composed of at least 30% of both glandular and neuroendocrine carcinoma components.^{1,4,5} The histologic components of MiNEN should be individually graded.^{4,7} The present case was composed predominantly of large cell carcinoma (70%), as confirmed by immunohistochemical evaluation, admixed with well-differentiated adenocarcinoma (30%). Chronic inflammation and intestinal metaplasia were noted in the background.

Endocrine cells are ubiquitously seen throughout the gastrointestinal tract but are absent in the normal gallbladder except for a few cells in the neck region.¹¹ This explains the low prevalence of NENs in the gallbladder (2%). Although the origin of MiNENs remain unclear, it has been hypothesized that these tumors may have been derived from a single pluripotent stem cell precursor that is capable of divergent phenotypic differentiation.⁶ Another proposed mechanism is through metaplastic change. Gallbladder mucosa that underwent gastric and intestinal metaplasia, which are commonly associated with chronic cholecystitis and cholelithiasis, express different types of neuroendocrine cells.¹⁰ These cells are postulated to follow the metaplasia-dysplasia-carcinoma sequence. An alternative view is that MiNENs may have

arisen from the transdifferentiation of adenocarcinoma cells. Evidence also support the association of chronic inflammation to gallbladder cancer.^{4,5,11,12} The hypothesis that MiNEN arises from a common precursor stem cell that undergoes differentiation into several distinct phenotypes is supported by the finding of similar immunohistochemical and ultrastructural profiles in both carcinoma components.¹³ The detection of an identical molecular genetic alteration (TP53 missense mutation leading to a stop codon) in the individual components of the above case further supports the hypothesis that the tumor arises from a common progenitor cell.

p53 is a tumor suppressor gene located on chromosome 17p, the phosphoprotein product of which is involved in the regulation of cell division, by acting as a transcription factor that modulates cyclin-dependent kinase activity. p53 gene mutations are the most frequent genetic abnormality in human cancers. Mutations can be detected using immunohistochemical staining of the p53 protein product or molecular studies such as somatic mutation profiling. In gallbladder cancers, high grade neoplasms exhibit a greater p53 positivity as compared with low grade tumors, and immunoreactivity to p53 might be associated with a shorter patient survival.¹⁴ A case of combined large cell neuroendocrine carcinoma and adenocarcinoma of the gallbladder displaying p53 overexpression and high Ki67 proliferative index was previously reported.¹⁵ Next generation sequencing of 15 gallbladder cancer cases, including adenocarcinoma, adenosquamous carcinoma and carcinosarcoma, also revealed that P53 mutations are the most common of the 26 mutations identified. Other mutations involved the following genes: TP53, STK11, CCNE1, MDM2, MYC, RICTOR, APC, ARID1A, AURKA, CDKN2A, CDKN2A/B, CRKL, FGF10, FGFR3-TACC, KRAS, MCL1, PPKARIA, SMAD4, SMARCA4, TSC2, BAP1, ERBB2, PIK3CA, and ZNF703.¹⁶

Acosta et al.,⁴ reviewed and summarized biliary MiNEN cases reported in the literature. Patients are diagnosed at a mean age of 64 years. The tumor is more commonly seen in women (female to male ratio of 2:1) and in Asian patients, with nonspecific epigastric or right upper quadrant abdominal pain as the most common presenting symptom. Two-thirds of biliary MANEC cases primarily arise from the gallbladder. Patients usually have locally advanced disease (T3 in more than 60%) and lymph node metastasis (half of the cases) at initial diagnosis. Majority of the cases reported were treated with surgery alone, others were treated with chemotherapy and/or radiotherapy.

Most gallbladder carcinomas (GBCs) are both clinically and radiologically unapparent as they mimic presentations of benign diseases such as cholecystitis.⁵ The initial symptoms of primary GBCs are nonspecific and as previously mentioned, patients most commonly present with right upper quadrant or epigastric pain. Histologic typing of GBCs and MiNENs is of utmost important since the treatment is tailored to the most aggressive component present in the tumor.^{4,8} Complete en-bloc surgical resection is the only curative treatment modality in GBCs. Patients with MiNEN generally fare better than those diagnosed with a pure biliary PDNEC.⁸ However, despite adequate surgical management, the

prognosis of biliary MiNENs remain generally poor and this is partly attributed to the delay in their diagnosis and treatment.^{1,4,8,10,12} Tumor recurrence is highly considered for this patient, hence, close clinical follow-up and monitoring is vital for prompt management.

CONCLUSION

We report a case of a 74-year-old Filipino woman who was diagnosed with MiNEN composed of a well-differentiated adenocarcinoma and a large cell neuroendocrine carcinoma component. Molecular analysis of the respective components revealed a similar molecular signature, confirming the common/monoclonal origin hypothesis and indicating that this entity is most likely derived from a pluripotent stem cell capable of divergent differentiation.

ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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