Large cell neuroendocrine carcinoma (LCNEC) of the gallbladder is an extremely rare malignancy, comprising 2% of all gallbladder cancers.1 The prognosis of this tumor is poor and early metastasis is common.2 To date, 18 cases of gallbladder LCNEC have been reported.3,4 LCNEC of the gallbladder is usually combined with other types of malignancies, mostly adenocarcinoma. We present an unusual case of combined adenosquamous carcinoma (ASC) and LCNEC of the gallbladder.

CASE REPORT

A 54-year-old woman presented with a 4-month history of intermittent epigastric pain. Laboratory tests revealed elevated serum levels of alkaline phosphatase and gamma-glutamyltransferase, up to 161 IU/L (reference range, 30 to 120 IU/L) and 81 IU/L (reference range, 9 to 64 IU/L), respectively. The levels of other liver enzymes including aspartate aminotransferase, alanine aminotransferase, as well as total bilirubin and direct bilirubin levels, were within normal range. Serum carbohydrate-associated antigen (CA19-9) was 151.4 IU/mL (normal < 37 IU/mL), and α-fetoprotein and carcinoembryonic antigen were within normal range. Abdominal computed tomography showed a 6.9 cm mass in the gallbladder fundus, with direct invasion of the adjacent liver (Fig. 1A). Biopsy of the liver mass showed poorly differentiated carcinoma with extensive squamous differentiation. Radical cholecystectomy was performed and gross examination revealed a 4.3 × 4.0 cm polypoid mass in the fundus with infiltration of adjacent liver parenchyma and multiple yellowish gallstones (Fig. 1B, C). An incidentally found 0.4-cm-sized nodule was also noted in the liver.

Microscopically, the tumor comprised two distinct components: ASC and LCNEC. The adenocarcinoma (AC) component of the ASC consisted of variable sized glands, lined by atypical columnar epithelium. The squamous cell carcinoma (SCC) component showed atypical stratified squamous epithelium with occasional keratin pearls. An abrupt transition from AC to SCC was observed (Fig. 2A, B). In addition, there was an area of LCNEC consisting of round cells with large, vesicular nuclei and frequent mitotic figures (33/10 high-power fields). Occasional rosette formation was found. The tumor cells were arranged in trabecular, palisad-
ing, or solid patterns, and mixed with an AC component in the periphery (Fig. 2C, D). These cells showed diffuse and strong positivity for synaptophysin and chromogranin, and focal positivity for CD56 immunohistochemistry (Fig. 3A–C). The Ki-67 proliferating labeling index was 25% (Fig. 3D). The tumor was largely composed of ASC, and the proportion of the SCC component was approximately 80%, whereas the area of LCNEC was small, constituting approximately 10% of the entire tumor. The gallbladder wall was mainly composed of AC components, while the proportion of SCC and LCNEC components increased substantially as the tumor invaded into the liver parenchyma. Multifocal lymphovascular invasion by SCC and perineural tumor invasion by LCNEC were identified. Only a few foci of lymphovascular invasion were observed with an AC component. Chronic cholecystitis with intestinal metaplasia were identified in the remaining mucosa. No metastasis was present in the seven regional

**Fig. 1.** Imaging and gross findings. (A) On abdominal computed tomography, the tumor of the gallbladder shows direct invasion of the adjacent liver. (B) On opening the gallbladder, a fungating mass is observed in the fundus. (C) The cut surface shows a relatively well-circumscribed, yellowish fibrotic mass, with hemorrhage and necrosis, invading the liver parenchyma.

**Fig. 2.** Microscopic findings. (A, B) Adenosquamous carcinoma component consisted of variable-sized glands lined by atypical columnar epithelium (left) and atypical stratified squamous epithelium (right). (C) Neuroendocrine carcinoma component, transition from adenocarcinomatous component (left) and composed of large cells arranged in solid sheets (right). (D) The neuroendocrine component shows solid and pseudorosette patterns.
lymph nodes. The metastatic nodule in the liver was composed purely of an LCNEC component. The final diagnosis of the gallbladder was confirmed as combined ASC and LCNEC. The patient received adjuvant chemotherapy post-operatively. However, magnetic resonance imaging revealed two liver metastases at the 3-month follow-up. Wedge resection of the liver was performed on the metastatic nodules, measuring 4.7 cm and 3.4 cm. Microscopically, the metastatic nodules were composed purely of an SCC component with no AC or LCNEC components. The patient was treated with chemotherapy (gemcitabine, cisplatin, and epirubicin) and radiotherapy after the operation, and died after 13 months from the initial diagnosis.

This study was approved by the Institutional Review Board of Korea University Anam Hospital, and informed consent was waived (ED17132).

**DISCUSSION**

The majority of carcinomas in the gallbladder are ACs, comprising up to 88%. Other carcinomas include ASC (5%), neuroendocrine carcinoma (NEC) (2%), SCC (1%), and undifferentiated carcinoma (< 1%). Identifying the exact histologic component of the tumor is important because the histologic type is associated with patient prognosis in gallbladder cancers. ACs have a relatively favorable prognosis, whereas tumors with squamous or neuroendocrine differentiation have a poor outcome (mean survival time, 50 vs 23 or 10 months, respectively). LCNEC has been known to exhibit aggressive behavior and early metastasis. Among the previously reported LCNEC cases, 53% had directly invaded the liver at the time of diagnosis, and 47% had been identified with locoregional or distant metastases involving bone, liver or lymph nodes. In this case, the tumor showed direct invasion of the liver parenchyma at the time of initial diagnosis and additional multiple liver metastases afterwards, demonstrating aggressive behavior.

According to the recent 2010 World Health Organization classification, neoplasms, morphologically composed of both glandular epithelial cells and neuroendocrine cells with over 30% of each component are described as mixed adenoneuroendocrine carcinoma (MANEC). The present case was predominantly composed of SCC (about 80%) and the amount of AC or NEC component was not enough for the criteria of MANEC.
The histogenesis of NEC in the gallbladder is still not clear, because neuroendocrine cells are not normally present in the gallbladder except for a few cells in the neck region. It has been postulated that they may arise from pluripotent precursor cells.8 However, the pathogenesis of differentiation from stem cells to neuroendocrine cells remains unclear. Another hypothesis for the development of NEC of the gallbladder is through metaplastic change.9 Intestinal or gastric metaplasia occurs as a result of cholelithiasis and chronic inflammation.10 Argentaffin cells have been identified in the metaplastic mucosa and these cells have been suggested as precursors of neuroendocrine tumor in the gallbladder.11 Almost all previously reported gallbladder LCNECs described coexisting cholelithiasis with chronic cholecystitis.7 In this case, chronic cholecystitis with intestinal metaplasia associated with cholelithiasis was identified, which suggested a precursor lesion of the NEC.

It is noteworthy that metastatic nodules show two different histologic types. The incidentally identified, synchronous metastatic hepatic nodule was totally composed of an LCNEC component, whereas the metachronous metastatic nodules were composed entirely of an SCC component. The metastatic potential is different in each type of cancer cells.12 The SCC component has higher proliferative activity than the AC component and more frequently observed in the invasive front of the tumor and in lymphovascular and perineural invasion in the gallbladder ASC.13 This might contribute to the metastatic potential and tumor progression in ASC.13 In the present case, the SCC component was frequently observed in lymphovascular invasion and caused multiple pure SCC metastatic nodules. Additionally, LCNEC has higher risk of metastasis through lymphatic invasion, compared to the AC.14 Acosta and Wiley15 reported that the majority of lymph node metastases shows only NEC components in primary MANECs of the biliary tree. This case is consistent with previous studies in that the metastatic nodules were composed of either SCC or NEC, without an AC component, and this might contribute to the metastatic potential and poor prognosis of ASC and NEC of the gallbladder.

There are also differences in chemotherapeutic agents between conventional AC and combined tumor of the gallbladder. Conventional AC is usually treated with gemcitabine and cisplatin, while combined ASC or NEC is treated with FOLFOX (leucovorin, fluorouracil, and oxaliplatin) or etoposide.1,2

This is an unusual case of LCNEC combined with ASC. The histologic types of the gallbladder carcinoma might contribute to the metastatic potential and poor prognosis. To detect an LCNEC or ASC component is important because the tumor may require a different chemotherapy regimen and show early metastasis with poor prognosis.

Conflicts of Interest
No potential conflict of interest relevant to this article was reported.

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