Case Report
Mixed small cell neuroendocrine carcinoma and adenocarcinoma of the gallbladder: a case report

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Abstract: Objective: To investigate the clinical features and treatment options of mixed small cell neuroendocrine carcinoma and adenocarcinoma of the gallbladder. Methods: Gallbladder small cell carcinoma comprises only 0.5% of all gallbladder cancer. It is composed of invasive tumors and the effect of existing treatments is poor. Here, we report a case of small cell neuroendocrine carcinoma with a mixed gallbladder adenocarcinoma. Results: There were no specific symptoms or abnormal blood test results in this case. Imaging examinations indicated that an intraluminal mass in the gallbladder was accompanied by multiple microlithiasis, and the lymph node was enlarged in the hepatic hilum. The mass then oppressed the bile duct, and jaundice appeared. Radical hepatopancreaticoduodenectomy (HPD) was performed. Pathological diagnosis was a mixture of small cell neuroendocrine carcinoma (65%) and adenocarcinoma (35%) of the gallbladder. The TNM stage was IVB, T3N2M0. Four courses of postoperative chemotherapy were carried. Twenty-five weeks after surgery, brain metastasis appeared, and local palliative three-dimensional adaptive radiotherapy was performed. The patient died 28 weeks after surgery. Conclusion: Gallbladder small cell neuroendocrine carcinoma (SCNEC) is a highly malignant disease. Radical surgery and other comprehensive treatment with combinational chemotherapy might improve the prognosis.

Keywords: Small cell neuroendocrine carcinoma, adenocarcinoma, mixed adenoneuroendocrine carcinoma, gallbladder, hepatopancreatoduodenectomy

Introduction
Neuroendocrine neoplasms (NETs) are tumors that originate from neuroendocrine cells throughout the body, most commonly in the lung and gastrointestinal tract. The proportion of neuroendocrine carcinoma in gallbladder cancer is less than 2% [1]. Primary gallbladder NETs are rare, accounting for 0.2% [2]. Neuroendocrine neoplasms of the gallbladder (GB) are rare tumors classified as grade 1 and 2 neuroendocrine tumor (NET), neuroendocrine carcinoma (large cell or small cell type), and mixed adenoneuroendocrine carcinoma (MANEC) [3]. Small cell neuroendocrine carcinoma (SNEC) is a highly malignant tumor characterized by hyperplasia of the nucleus, sparse cytoplasm and high nuclear/cytoplasmic ratio [4]. Almost all patients with gallbladder neuroendocrine carcinoma (GNEC) were diagnosed incidentally on the basis of pathological examination and postoperative immunohistochemical staining [5]. Due to limitations of the cases, the clinical features and prognosis of GNEC remain uncertain. Here, we report a case of gallbladder carcinoma with pathological confirmation of MANEC. The patient survived for 28 weeks after radical surgery, postoperative chemotherapy, and three-dimensional radiotherapy. This case might help recognize the clinical features and treatment options of this disease.

Case report
An incidental mass in the gallbladder was revealed in a 52-year-old woman during abdominal ultrasound screening. She was asymptomatic and showed no evidence of jaundice. Routine laboratory tests, including liver function tests and tumor markers (CEA, AFP, CA199, CA125, CA724), were unremarkable. Abdominal B-ultrasound showed an intra-luminal mass and microlithiasis in the gallbladder and multiple enlarged lymph nodes in the hepatic portal...
Figure 1. Imaging examinations of patients. A: Preoperative abdominal ultrasound (an intra-luminal mass and several microlithiasis in the gallbladder, multiple enlarged lymph nodes in the hepatic portal area; single arrow denotes enlarged lymph nodes, and double arrow indicates tumor); B: Preoperative abdominal CT scan (a mass in the neck of the gallbladder, lymph nodes enlargement around the hepatic hilum, the lymph nodes around the head of the pancreas were fused into groups; single arrow denotes enlarged lymph nodes, and double arrow indicates tumor).

Figure 2. Hematoxylin-eosin staining and immunohistochemistry specimens of MANEC specimens. A: Hematoxylin-eosin staining of MANEC (SCNEC accounted for 65%, adenocarcinoma, 35%; original magnification 100×); B: Syn (partial cells (+++); original magnification 100×); C: CgA (partial cells (+); original magnification 100×); D: CD56 (++; original magnification 100×); E: CK7 (+; original magnification 100×); F: Ki67 (about 50% (+); original magnification 100×; arrows indicate positive protein, brownish or tan.)

Abdominal computed tomography (CT) scans showed a mass in the neck of the gallbladder, with lymph node enlargement around the hepatic hilum (Figure 1A). The lymph nodes around the head of the pancreas were also fused into groups and there were no signs of distal metastases in CT scans of the chest, the liver, kidneys, and pelvis. Hepatopancreatoduodenectomy (HPD) was performed on May 25th, 2011 under general anesthesia. No distal metastasis or ascites was observed during the operation. The size of gallbladder was about 12×5×6 cm, high tension. The size of the intraluminal mass in the gallbladder neck was about 7.5×6.5×5 cm. The whole layer of the gallbladder wall was infiltrated. Multiple enlarged lymph nodes were in hepatoduodenal ligament. The lymph nodes around the pancreatic head merged into a lump, with the largest diameter being 4 cm. The nodes had a hard texture and were poorly moveable. Intraoperative diagnosis was gallbladder carcinoma. After lymph nodes dissection of the sixteenth group, rapid pathology prompted no carcinoma invasion. The hepatic duct was cut at the junction of the left and right hepatic duct. We removed the upper margin of the bile duct, and rapid pathology prompted no carcinoma invasion. HPD (S4b, S5 segmentectomy of the liver + cholecystectomy + the pancreas head, gastric pyloric sinus, duodenum, and common bile duct and regional lymph nodes dissection) was performed. Routine digestive tract reconstruction lasted 8 hours and the bleeding volume was 1,000 ml. Postoperative pathology reported Gallbladder MANEC (Figure 2A), SCNEC accounted for 65%, adenocarcinoma, 35%. Some adenocarcinoma types were
mucous adenocarcinoma, while the rest were differentiated adenocarcinoma (sieving, tubular, or papillary). The lymph nodes around the pancreatic head and the gallbladder were metastatic. The TNM stage was IVB, T3N2cM0. Immunohistochemistry findings was as follows:

Syn partial cells (++++) (Figure 2B), TTF1 (++~+++), CgA partial cells (+) (Figure 2C), and CD56 cells (++) (Figure 2D). CDX-2, CK7 (Figure 2E), and Villin all showed adenocarcinoma (+). CK20 showed mucinous adenocarcinoma (+), and EGFR very few cells (+). The Ki67 index of three carcinomas was about 50% (+) (Figure 2F), P53 (+++), COX-2 adenocarcinoma (++~-+++), CerbB2 adenocarcinoma (++~+++), VEGF adenocarcinoma (+++). The gastric tube was removed on the 7th day after surgery. On the 9th day, the case began to have a liquid diet and a semi-liquid diet on the 12th day. In the first course, GEMOX (gixitabine + oxaliplatin) chemotherapy began on July 28th, 2011, followed by 3 degrees of alimentary canal reaction after chemotherapy, and two courses of “peierus and sodium + nitozumab” chemotherapy were given on August 29th, 2011 and September 22nd, 2011. On October 12th, 2011, liver and retroperitoneal lymph nodes metastasis was found (Figures 3A and 3B). After October 13th, 2011, the course of “nitozumab and albumin combined with taxol” chemotherapy was given. On November 9th, 2011, CT scan indicated a significant reduction in intrahepatic tumors (Figures 4A and 4B). On November 18th, 2011, the patient appeared to have a weak right limb, and muscle strength was 1 degree, the right barbinski sign (+), and the left muscular force was 4 degrees. Magnetic resonance imaging (MRI) of the head showed multiple intracranial occupying, considering brain metastasis (Figures 5A and 5B). Three-dimensional adaptive radiotherapy was given on December 1st, 2011, and the cumulative dose was 14 Gy/7 f. The second
radiotherapy was given on December 15th, 2011, and the cumulative dose was 22 Gy/11 f. There was no benefit for the patient, and treatment was ceased. The patient died 28 weeks after surgery.

Discussion

The origin of neuroendocrine carcinoma of the gallbladder is still controversial, because the neuroendocrine cells are widely distributed in the gastrointestinal tract. These tumors are identified from gastrointestinal pluripotent stem cells, rather than other parts of the cell migration, since no neuroendocrine cells exist in the gallbladder [6]. Some scholars believe that GNEC is derived from metaplastic gallbladder mucosal cells. Sakamoto et al. studied 103 cases of gallbladder resection due to cholecystitis and found that 12 cases (11.7%) of gallbladder were with intestinal metaplasia [7]. In cases of intestinal metaplasia in the gallbladder, the expression rates of chromogranin A (CgA or CHG) and serotonin were 83.3% and 50.0%, respectively. The two types of protein are expressed by the neuroendocrine cells, further supporting this view. Other scholars have proven that gastrointestinal neuroendocrine and adenocarcinoma can be converted to each other [8]. Under normal conditions, gallbladder malignant lesions are almost always adenocarcinomas. Neuroendocrine carcinoma accounts for only 1.25% of all malignant tumors. The diagnosis of GNEC is based on the 2010 World Health Organization (WHO) classification [9]. The classification of neuroendocrine carcinomas is classified according to the WHO classification and European Neuroendocrine Tumors classification system [10]. The diagnostic criteria of gallbladder SCNEC is as follows: (i) More than one type of protein, including CgA and Syn, or CD56 immunohistochemical staining was positive, or indicating the presence of neural cell adhesion molecules; (ii) In histopathology and cytogenetics, there are significant small cell features, nuclei mostly stained, few nucleoli or no nucleoli, and sparse cytoplasm, circular or fusiform [11-13]. However, the clinical manifestations and imaging testing results such as B-ultrasound and CT examination for gallbladder neuroendocrine carcinoma lack specificity and are not typical. Therefore, the diagnosis is very difficult to draw before surgery and pathological examination is the golden rule for diagnosis.

Patients of gallbladder neuroendocrine cell carcinoma mixed with adenocarcinoma are very rare, with no more than 150 cases reported in the literature [14]. Cholecystectomy is adequate if gallbladder specimens only show intramucosal cancers, or pathologic reports reveal a single cancer with mucosal or submucosal invasion only (T1) [15]. However, in patients with advanced non-distant metastases, radical cholecystectomy and lymph node dissection should be performed in combination with hepatectomy [16]. Improved results have been achieved in aggressive radical surgery for gallbladder carcinoma and some reports suggest that patients with locally invasive gallbladder neuroendocrine carcinoma may benefit from aggressive surgeries followed by adjuvant chemotherapy [17]. Clinical studies have shown that HPD is the only surgical modality for R0 resection for diffuse cholangiocarcinoma. Surgical indications include tumor directly invading the pancreas, duodenum, or lymph node metastasis of the pancreas and duodenal area. But because of the malignant potency of neuroendocrine carcinoma, it has the characteristics of early lymph node metastases, fatal vessel invasion and distant metastases. Poor prognosis still exists even after resection. Systemic chemotherapy is the preferred treatment [18]. According to Epidemiological and End Results (SEER) data, the 1-year survival rate and 5-year survival rate of the gallbladder SCNEC is 21% and 0%, respectively [19]. The postoperative survival period of the two mixed tumor types was approximately 4 months, and rarely exceeded 1 year, which remains a tough problem for surgeons [20]. In terms of prognosis, tumor infiltration of adjacent structures is an important predictor of prognosis compared with the prognosis of localized gallbladder wall tumors [21]. Evidence of elevated Ki67 and mitotic index may also predict poor prognosis, as reported in other neuroendocrine carcinomas [22]. Moskal et al. reported that the most common metastatic sites of gallbladder SCNEC were lymph node (88%), liver (88%), lung (23%) and peritoneum (19%) [23]. Despite the poor prognosis of the gallbladder SCNEC, previous studies have reported that aggressive multimodal treatment may prolong survival [24].
In our present study, we encountered a case with small cell neuroendocrine carcinoma combined with adenocarcinoma in the gallbladder. B-ultrasound and abdominal computed tomography scans showed an intraluminal mass with multiple small stones in the gallbladder and lymph nodes enlargement in the hepatic hilum. Lymph nodes around the pancreatic head merged into a mass. The mass had a compressed common bile duct. We adopted aggressive surgical methods. During the surgery, we dissected the abdominal aorta lymph nodes (the 16th group of lymph nodes), and rapid pathology prompted no carcinoma invasion. We cut the common bile duct at the junction of the left and right hepatic duct. We removed a cycle of bile duct tissue and rapid pathology prompted no carcinoma invasion, too. HPD treatment was then performed. The regional lymph nodes dissection contained the hepatoduodenal ligament regional lymph nodes and the lymph nodes around the posterior of the duodenum and the upper edge of the pancreas and the lymph nodes around the head of the pancreas. Conventional gastrointestinal reconstruction was performed. Immunohistochemistry staining of tumor samples showed neuroendocrine carcinoma expression: syn part cells (+++), TTF1 (+~++), CgA part cells (+), CD56 part cells (+), CDX-2, CK7 and Villin showed adenocarcinoma (+), CK20 showed mucinous adenocarcinoma (+), EGFR very few cells (+). The case was in accordance with mixed adenoneuroendocrine carcinoma. The gallbladder carcinoma broke through the gallbladder wall and invaded adjacent structures. The pathological stage: IVB (T3, N2, cM0) was late. Immunohistochemistry showed 50% of Ki67 cancer cells and prognosis was poor. After surgery, 4 courses of postoperative chemotherapy were carried out. Two courses of brain palliative three-dimensional adaptive radiotherapies were given after the brain metastases had been found. However, the patient died at 28 weeks after surgery. The effectiveness and feasibility of treatment in patients with both neuroendocrine and adenocarcinoma in the gallbladder needs to be further examined in the larger number of cases. By introducing this patient, we believe a combination of early diagnosis, surgical treatment and proper postoperative chemotherapy may contribute to a better prognosis. An increased awareness of treatment choices and biological feature of MANEC is urgently required.

In conclusion, gallbladder neuroendocrine carcinoma, an aggressive and poor prognosis malignancy, is a rare subtype of gallbladder tumor. For patients with no distant metastasis, a radical surgery is feasible and adjuvant chemotherapy and radiotherapy is combined. There are no specific methods to treat GNEC. We need to strengthen regular medical examinations for early diagnosis and conduct more large scale prospective studies in the future.

Disclosure of conflict of interest

None.

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