

# A Case of Combined Hepatocellular and Cholangiocarcinoma with Neuroendocrine Differentiation and Sarcomatoid Transformation

## - A Case Report -

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We report here on a case of combined hepatocellular and cholangiocarcinoma (CHC) with neuroendocrine differentiation and sarcomatoid transformation. A 59-year-old male who had had HBV-associated chronic liver disease presented with hepatic masses. The explanted liver showed three small masses, two in the right lobe and one in the left lobe. The largest one in the right lobe was a 2.0 cm sized binodular mass, consisting of a yellowish tan nodule and an abutting reddish brown nodule. Microscopically, the reddish brown nodule was a cholangiocarcinoma (CC) showing neuroendocrine differentiation and sarcomatoid transformation. The yellowish tan nodule and the remaining two masses were hepatocellular carcinoma (HCC). On immunohistochemistry, both the adenocarcinoma and spindle sarcomatoid cells were positive for pancytokeratin, but only the adenocarcinoma cells were positive for chromogranin and carcinoembryonic antigen (CEA). Mitotic and Ki67 labeling indices as well as p53 immunopositivity were significantly increased only in the CC component. We report here on the first case of CHC in which the CC displayed neuroendocrine differentiation and sarcomatoid transformation with high mitotic and Ki67-labeling indices, as well as having p53 overexpression.

**Key Words** : Carcinoma; Hepatocellular-Cholangiocarcinoma; Ki67 Antigen; Protein p53; Neuroendocrine differentiation; Sarcomatoid transformation

It is known that hepatocellular carcinoma (HCC) can exhibit various histologic features even in the same cancerous nodule, and these features can be a neuroendocrine differentiation or sarcomatoid transformation, and HCC can be observed in combination with cholangiocarcinoma (CC). Combined hepatocellular and cholangiocarcinoma (CHC) is a rare neoplasm, and it has more frequent nodal metastases and a lower 5-year survival rate, leading CHC to have a worse prognosis than HCC.<sup>1</sup> Areas of sarcomatoid transformation are found in 2-4% of all HCCs, but CHCs with sarcomatoid transformation are extremely rare.<sup>2-7</sup> Sarcomatoid transformation usually indicates a biphasic tumor with a carcinoma component and homologous spindle sarcomatoid component, but this sarcomatoid transformation is rarely associated with heterologous components having rhabdomyoblastic, osteoblastic, or giant cell differentiations.<sup>3-5</sup> Neuroendocrine differentiation has been uncommonly reported in poorly or undifferentiated primary hepatic neoplasia.<sup>8-10</sup> The implication of the neuroendocrine differentiation has not yet been elucidated,<sup>8</sup> however, primary neuroendocrine tumors of the liver have been reported to metastasize earlier.<sup>9,10</sup> There have been no reported cases of

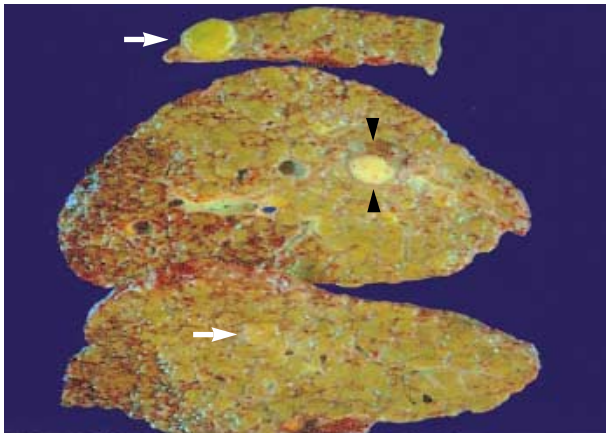
CHC with neuroendocrine differentiation and sarcomatoid transformation in the English literature.

We describe here on the first reported case of CHC with neuroendocrine differentiation and sarcomatoid transformation.

## CASE REPORT

A 59 year-old male presented with hepatic masses that were found during a work-up for his indigestion that had been begun eight months previously. He'd had hepatitis B virus (HBV) associated chronic liver disease that had been diagnosed 13 years ago. He was a heavy alcoholic, however, the other parts of his past medical history were unremarkable. In his family history, his two elder brothers died of liver disease. The physical exam findings were unremarkable, except for his mild abdominal distension. On admission, the abnormal laboratory findings were as follows: sGOT, 90 IU/L; sGPT, 111 IU/L; alkaline phosphatase, 203 IU/L;  $\gamma$ -GT, 120 IU/L; and alpha-fetoprotein (AFP), 68.4 ng/mL. The serum level of carcinoembryonic antigen (CEA) was normal. The

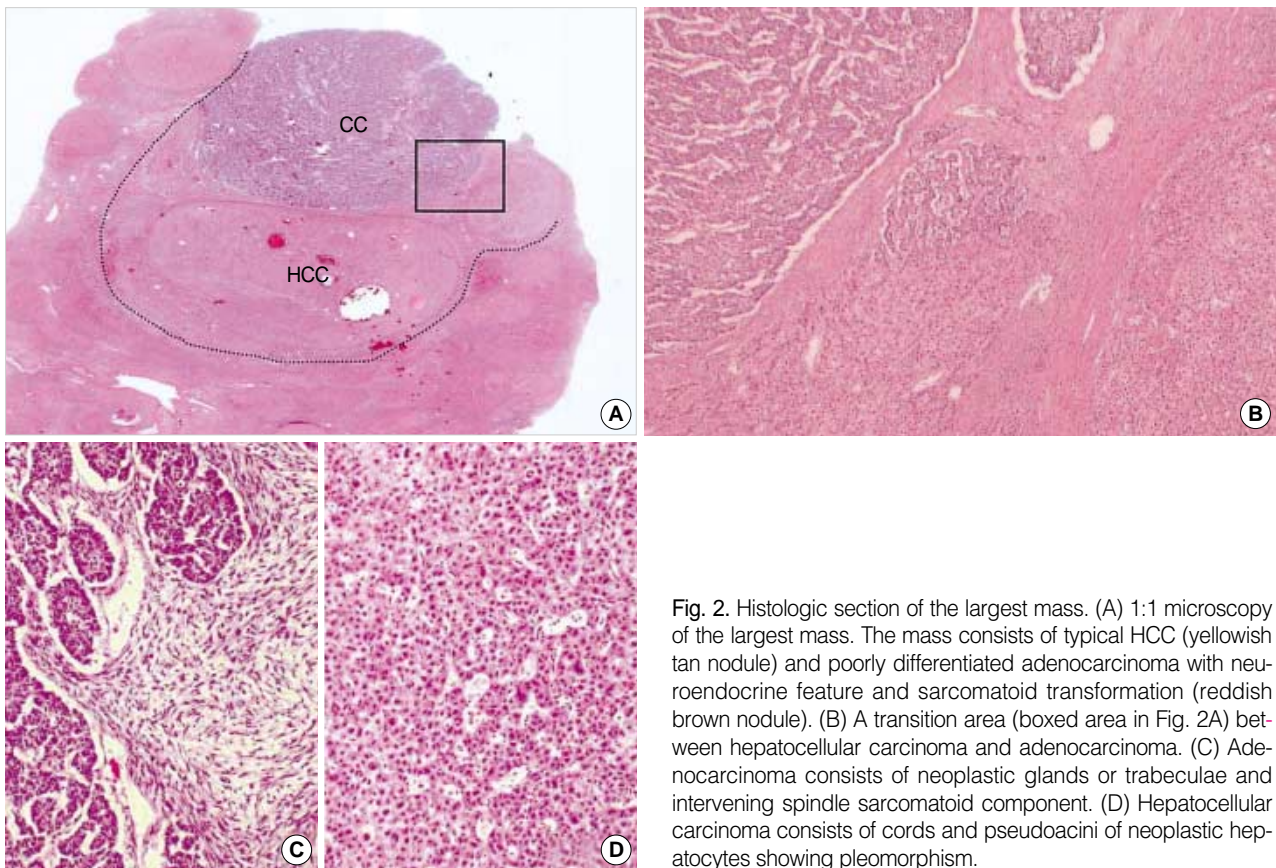
viral markers for hepatitis B surface antigen (HBsAg) and hepatitis B envelope antigen (HBeAg) were positive. Computed tomographic scan revealed three arterial enhancing masses in the cirrhotic liver. After four treatments of transarterial chemoembolization, a living donor liver transplantation was performed.



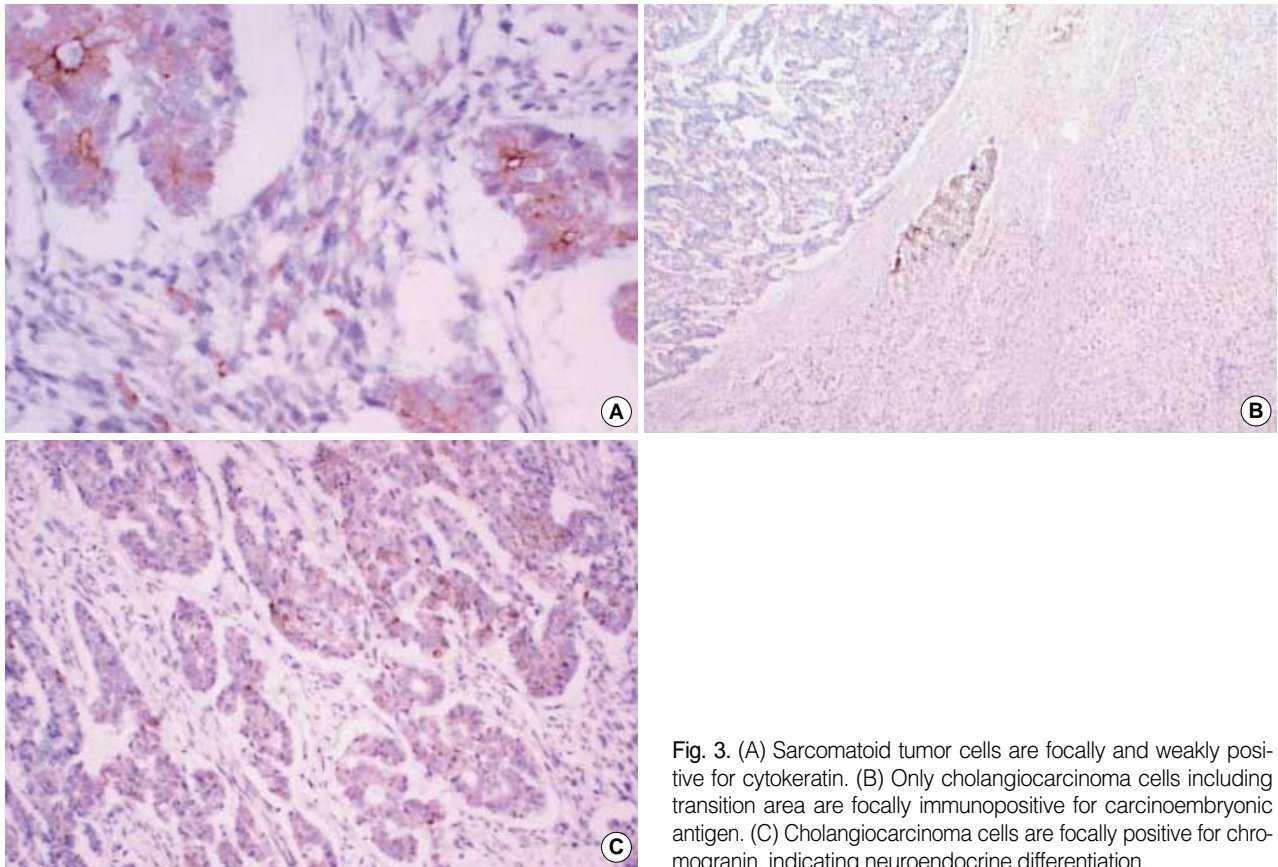
**Fig. 1.** The resected liver shows three small and separate masses. The largest mass in the mid-portion (arrow heads) is binodular, consisting of a yellowish tan nodule and an abutting reddish brown nodule. The other two masses (arrows) are yellowish green and soft without hemorrhage or necrosis.

The resected liver showed three separate masses. There were two well-defined masses ( $2.0 \times 1.4 \times 1.0$  cm and  $1.2 \times 1.0 \times 0.5$  cm) in the right lobe and the other mass ( $1.7 \times 1.7 \times 1.0$  cm) was in the left lobe. Upon gross examination, the largest mass was soft to firm and binodular with yellowish tan areas and abutting reddish brown areas without hemorrhage or necrosis. The other two masses were yellowish green and soft with no hemorrhage or necrosis. The remaining hepatic parenchyma was cirrhotic (Fig. 1).

Microscopically, the largest mass consisted of HCC (the yellowish tan nodule) and poorly differentiated adenocarcinoma with neuroendocrine features and sarcomatoid transformation (the reddish brown nodule) (Fig. 2A). There was an area of transition between HCC and adenocarcinoma (Fig. 2B). The adenocarcinoma consisted of neoplastic glands or trabeculae separated by intervening spindle sarcomatoid components (Fig. 2C). The neoplastic glands consisted of polygonal cells showing eosinophilic granular cytoplasm. On d-PAS staining, the adenocarcinoma cells demonstrated intraluminal mucin, however, the tumor cells were negative for mucicarmine. The sarcomatoid area was entirely composed of pleomorphic spindle cells. Mitotic figures were markedly increased in both the adenocarcinomatous and



**Fig. 2.** Histologic section of the largest mass. (A) 1:1 microscopy of the largest mass. The mass consists of typical HCC (yellowish tan nodule) and poorly differentiated adenocarcinoma with neuroendocrine feature and sarcomatoid transformation (reddish brown nodule). (B) A transition area (boxed area in Fig. 2A) between hepatocellular carcinoma and adenocarcinoma. (C) Adenocarcinoma consists of neoplastic glands or trabeculae and intervening spindle sarcomatoid component. (D) Hepatocellular carcinoma consists of cords and pseudoacini of neoplastic hepatocytes showing pleomorphism.



**Fig. 3.** (A) Sarcomatoid tumor cells are focally and weakly positive for cytokeratin. (B) Only cholangiocarcinoma cells including transition area are focally immunopositive for carcinoembryonic antigen. (C) Cholangiocarcinoma cells are focally positive for chromogranin, indicating neuroendocrine differentiation.

**Table 1.** Summary of the immunohistochemical findings in the present case

	Cirrhotic parenchyma	HCC	Cholangiocarcinoma and transition area	Sarcomatoid area
CK AE1/AE3	Negative	Negative	Positive (strong)	Positive (weak)
CK7	Negative	Negative	Negative	Negative
CK20	Negative	Negative	Negative	Negative
CEA	Negative	Negative	Positive (focal)	Negative
Hepatocyte antigen	Positive	Positive	Negative	Negative
AFP	Negative	Negative	Negative	Negative
Chromogranin	Negative	Negative	Positive (focal)	Negative
Synaptophysin	Negative	Negative	Positive	Negative
Ki67-positive cells	Rarely positive	15%	60%	30%
p53-positive cells	Rarely positive	22%	80%	48%

HCC, hepatocellular carcinoma; CK, cytokeratin; CEA, carcinoembryonic antigen; AFP, alpha-fetoprotein.

sarcomatoid areas. The HCC area was a typical HCC, Edmondson-Steiner grade 3 (Fig. 2D), and it exhibited considerable nuclear pleomorphism. Mitotic figures were not infrequently observed

in the HCC area.

The immunohistochemical findings of the present case are summarized in Table 1. The adenocarcinoma cells were strongly immunopositive, however, the spindle sarcomatoid cells were weakly positive for pankeratin and AE1/AE3 (CK AE1/AE3; 1:200, Zymed, San Francisco, CA, USA) (Fig. 3A). Both areas were negative for CK7 (1:200, DAKO, Glostrup, Denmark) and CK20 (1:200, DAKO). Immunoreactivity for CEA (1:400, Dinona, Seoul, Korea) was focally observed only in the adenocarcinoma area (Fig. 3B), while hepatocyte antigen (1:200, DAKO) was expressed only in the HCC and the surrounding hepatic parenchyma. AFP (1:200, Novo, San Francisco, CA, USA) was not expressed in both types of the tumor. The adenocarcinoma cells were focally positive for chromogranin (1:50, Dinona) (Fig. 3C) and they were only rarely positive for synaptophysin (1:100, Dinona), while the sarcomatoid tumor cells and HCC cells were not immunoreactive for both markers. The numbers of Ki67 (1:200, DAKO)-positive cells and p53 (1:1,600, DAKO)-positive cells were significantly increased in the adenocarcinoma area when compared with the HCC area (Table 1). However, the nonneoplastic hepatocytes were rarely positive for both markers. The remaining two masses were composed of typical HCC of Edmond-

son-Steiner grade 1 (tumor size, 1.7 cm in greatest dimension) and 3 (tumor size, 1.2 cm in greatest dimension) each. The patient is alive and well without tumor recurrence or metastasis at 25 months postoperatively.

## DISCUSSION

CHC has been subclassified as follows based on the presence of a transition area between the HCC and the CC and of the mucin-producing glands:<sup>11</sup> type I (collision tumors) has no transition areas between the HCC and CC; type II (transitional tumors) has areas of apparent transition between HCC and CC; and type III (fibrolamellar tumors) has tumor cells resembling a fibrolamellar variant of HCC, but the tumor contains mucin producing glands. According to this subclassification, our case corresponds to CHC, type II. Regarding the histogenesis, CHC has been thought to originate from intermediate cells or from progenitor cells having dual potential.<sup>12</sup> A genetic classification has been recently attempted based on the loss of heterozygosity patterns; this showed three possible forms: bichlonal neoplasms, single clonal neoplasms with homogeneous genetic changes, single clonal neoplasms with genetic progression/diversion in the HCC and/or the CC foci.<sup>13</sup>

Clinically, CHC is very much like HCC as it is associated with male predominance and chronic liver diseases of viral origins.<sup>1,12</sup> In addition, the AFP is occasionally elevated, but the CEA is rarely elevated in CHC. Thus, the CC component in the CHC cases has rarely been predicted before surgical resection, as was the situation in the present case.

The present case displayed neuroendocrine differentiation in the area of adenocarcinoma, and this area consisted of glands and trabeculae. In contrast to the HCC area, diffuse positivity for pankeratin, focal positivity for CEA and immunonegativity for hepatocyte antigen in the area of adenocarcinoma were all supportive evidences for the diagnosis of the CC component. Although CK7 is known as a marker of biliary differentiation and CC is usually positive for CK7, immunonegativity for CK7, as seen in this case, can be noted in CC. Instead, CK AE1 (specific for CK numbers 10, 14, 15, 16, 19) immunopositivity and hepatocyte antigen negativity were helpful for the differentiation between CC and HCC.<sup>14,15</sup>

Sarcomatoid transformation of primary liver cancers, including HCC and CC, has been reported with the incidence being 3.9%. However, only six cases of CHC with sarcomatoid transformation, including our case, have been reported.<sup>2-7</sup> The clinical

copathologic and immunohistochemical summaries of the previously reported cases are as follows. The patients' age ranged from 59 to 78 years (mean age, 66.3 years) and this tumor tended to affect males more frequently than females (male to female ratio 2:1). The tumors ranged in size from 2 cm to 21 cm in greatest dimensions (mean tumor size, 7.9 cm). The underlying livers were cirrhotic in all cases, however, viral markers were positive only in three cases including our case. Most of the sarcomatoid CHCs were grossly infiltrative, whitish gray, and necrotic. However, the present case was well demarcated, reddish brown, and soft to firm without hemorrhage or necrosis, which may be due to the very small size of the tumor. The sarcomatoid components showed undifferentiated, fibrosarcomatous or leiomyosarcomatous features. An osteoid component was seen in one case. Three cases showed sarcomatoid transformation only in the adenocarcinomatous area, while two cases showed sarcomatoid transformation in both the HCC and CC areas. The prognosis of liver cancers with sarcomatoid transformation is generally less favorable than that of ordinary HCC because of the more frequent nodal, intrahepatic and extrahepatic metastases.<sup>16</sup>

Primary neuroendocrine tumor of the liver in association with HCC, CC or fibrolamellar carcinoma has been infrequently reported.<sup>9</sup> Primary neuroendocrine tumor of the liver has been known to have a worse prognosis because of the early intrahepatic and nodal metastases.<sup>17</sup> In the present case, the focal, yet strong chromogranin immunoreactivity in the CC area represents the neuroendocrine differentiation. The relative proportion of cells that is needed to designate a tumor as 'neuroendocrine' has not been established, and the implication of neuroendocrine differentiation in ordinary adenocarcinoma is currently ill-defined. Therefore, a close follow-up is necessary in this type of case and more cases with neuroendocrine differentiation are required to define the significance of neuroendocrine differentiation in primary hepatic neoplasia.<sup>8</sup>

As a well-known marker of tumor progression, p53 overexpression was infrequently observed in the CC and HCC, and it is only rarely seen in CHC.<sup>5</sup> In the present case, a higher expression of p53 in the CC area may suggest a role for p53 in the transformation of tumor cells to the poorly differentiated adenocarcinoma. The Ki67 score (positive cells/total neoplastic cells) is a marker that seems to correlate with the Edmondson-Steiner histologic tumor grade, and it offers a useful information about the biological behavior of liver tumors.<sup>18</sup> In the present case, despite all the markedly increased p53 and Ki67 labeling indices as well as the increased mitotic activity, the patient is alive and well without recurrence or metastasis at 25 months follow-up. Therefore,

a long term follow-up is necessary to determine the prognosis of this patient. In conclusion, we report here a case of CHC with neuroendocrine differentiation and sarcomatoid transformation in the CC area. This is the first case of CHC having both neuroendocrine differentiation and sarcomatoid transformation which has not been described in the English literature.

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