Combined hepatocellular and cholangiocarcinoma with sarcomatous transformation was first recognized in Ramathibodi Hospital in 2005. This variant of carcinoma has been increasingly reported particularly from Asian countries. Dedifferentiation of the epithelial component to various sarcomatous components is likely the underlying mechanism. The causative factors of hepatocarcinogenesis in Thailand include chronic viral hepatitis B or C, exposures to aflatoxin B1 and nitrosamine(s) and occasionally some certain nodular hepatocellular lesions due to arterial hyperperfusion. It is suggested that the recent change of the Thai peoples’ life style to an increased consumption of fast foods containing food preservatives especially nitrate or nitrite, the nitrosamine precursor, may allow heavy exposure(s) to the chemical carcinogen(s) i.e. nitrosamine(s) leading to sarcomatous transformation of the carcinoma.

Keywords: Combined hepatocellular and cholangiocarcinoma, Sarcomatous transformation, Spindle-cell variant

Case Report

Hepatocellular carcinoma (HCC) with sarcomatous transformation has been variously referred to as malignant mixed tumor(1), carcinosarcoma(2), hepatoblastoma in adult(3), HCC with osteoclast-like giant cells(4,5), HCC with osteosarcoma or chondrosarcoma(5,9), HCC with sarcomatous change(9), spindle cell HCC or HCC with spindle cell change(6,10). The sarcomatous transformation has also been observed in intrahepatic cholangiocarcinoma (CGC)(11-13) and in combined hepatocellular and cholangiocarcinoma (HCC-CGC)(14-18).

In 2005, the authors encountered a case of HCC-CGC with sarcomatous transformation, which is the first case since the hospital’s foundation in 1969 as observed by one of us (SB). In the tumor periphery, small HCC cells and oval cells were observed in similarity to the authors’ recent observation of microscopic HCC-CGC(19). The recent recognition of sarcomatous transformation in Thailand seems to reflect a change in dietary pattern to an increased consumption of packaged or fast foods by Thai people in place of their regular home-cooked foods. This may allow heavy intake(s) of some certain food additive(s) acting also as carcinogenic precursor(s).

Case Report

The patient was a taxi motorcyclist, 28-year-old male, from Nakonsritamarat province presenting with a high fever and chill for 10 days prior to admission. The computerized-tomographic study (CT), done in another hospital, revealed a large septate cystic lesion 15 cm in diameter in the right liver lobe. The patient was then referred to Ramathibodi Hospital. His body temperature was 38.5°C. Laboratory investigation included hemoglobin 9.0 g/dl, hematocrit 29%, white blood cell count 14.4 x 10^3/µl, neutrophils 74%, lymphocytes 9%, monocytes 9%, basophils 1%, and...
platelet count 452 x 10^3/µl. Liver function tests included alkaline phosphatase 355 U/l (normal 50-160), gamma-glutamyltransferase 590 U/l (5-55), aspartate aminotransferase 72 U/l (15-37), alanine aminotransferase 117 U/l (30-65), total protein 73 g/l (64-82), albumin 42 g/l (43.1-53.3), total bilirubin 20.4 µmol/l (0-17.1), and direct bilirubin 10.2 µmol/l (0-5). Entamoeba histolytica titer was negative. HbsAg was positive.

Magnetic-resonance imaging study (MRI) disclosed one huge septate mass, 13.2 x 17.8 x 18.2 cm, occupying the entire right hepatic lobe (Fig. 1). Fluid-fluid level could be seen in the lower part of the lesion with loss of internal septation (Fig. 2). Preoperative diagnosis was hepatocellular carcinoma with necrosis. The exploratory laparotomy revealed a huge multiculated cystic mass containing 700 ml of serosanguinous fluid and necrotic tissue. The palliative resection of a large piece of liver tissue was performed.

The formalin-fixed liver specimen was 12 x 7 x 1 cm. The specimen was processed for light-microscopic examination by staining with hematoxylin and eosin (H and E), reticulin stain, Masson’s trichrome stain, and immunohistochemical stainings for BCL-2 protein (detecting protein protecting cells from apoptotic cell death due to withdrawal of the growth stimulating factors, Dako)(20), cyclin D1 (detecting elevations of cyclins in the G1-phase thereby allowing it to proceed to the S-phase G2-phase and mitotic phase, Dako), proliferative cell nuclear antigen and Ki 67 (detecting cyclin productions during G1- and G2- phases, Dako), glutathione S transferase-pi (detecting oncofetal expression, Immunotech), cytokeratin 7 (identifying bile duct cell, Dako), alpha-fetoprotein (identifying fetal hepatocytes and HCC, Dako), p53 protein (indicating the occurrence of mutation, Dako), S-100, vimentin, alpha-smooth muscle actin (detecting the mesenchymal expressions, Dako), and CD 117 (detecting tyrosine receptor in the cells of gastrointestinal stromal tumor, Dako). The results of immunohistochemical stainings are presented in Table 1.

Microscopically, the outermost part of the tumor consisted of small HCC cells frequently with binucleations (Fig. 3A). The tumor cells were arranged in two- to four-cell wide trabeculae separated by wide sinusoidal spaces. From the reticulin stain, reticulin fibers were of thick and thin fibers with an entangled network in the liver trabeculae (Fig. 3B). Focal absence of the HCC cells in the trabeculae could be seen (Fig. 3B). Transitional changes from HCC to ductular structures of CGC were observed in the inner zone of the tumor (Fig. 3C) consistent with the transitional type of HCC-CGC(8). The oval cell could be seen in proximity to the areas of small HCC cells (Fig. 3B)(19-21). They were strongly positive to the cytokeratin 7 immunostaining (Fig. 4A). Apoptosis of the ductular structures could be seen (Fig. 3C) more distinctively by the cytokeratin 7 immunostain (Fig. 4B), possibly due to the absence of BCL-2 production(19).

Inner to the mentioned zone, the regular sized HCC cells could be seen arranged in two- to four-cell trabeculae and occasional acinar pattern. Intratrabecular hemorrhages in the zone absent of HCC cells could be
Table 1. Results of the various immunohistochemical stainings

<table>
<thead>
<tr>
<th>Cell compartments</th>
<th>BCL-2</th>
<th>PCNA</th>
<th>Ki 67</th>
<th>GST-pi</th>
<th>CK 7</th>
<th>AFP</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small HCC cell</td>
<td>+</td>
<td>+++*</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malignant oval cell</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HCC-CGC cells</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- regular HCC cells in the trabecular or acinar pattern</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- duct cell</td>
<td>+&quot;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spindle HCC cell</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+&quot;</td>
<td>+&quot;</td>
<td>+&quot;</td>
</tr>
<tr>
<td>Spindle mesenchymal cell</td>
<td>+&quot;</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Giant tumor cell</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
</tbody>
</table>

Note:  
A. PCNA - proliferative cell nuclear antigen, GST-pi - glutathione S transferase-pi, CK 7 - cytokeratin 7, AFP - alpha-fetoprotein, Actin - alpha-smooth muscle actin  
b. p 53, S-100 and CD117 were negative in all cell components  
* +++ strongly positive, ± weakly positive  
= phenotypic expression in sporadic cells

Fig. 3  
A. a small HCC cell with binucleation (arrow)  
B. entangling reticulin network stained by reticulin stain, a part of the trabecula devoid of liver cell (arrow)  
C. small HCC cells in the left upper quadrant seen blending with bile-duct component of CGC in the right (arrow), apoptosis (small arrow). H and E x 400

Fig. 4  
A. the oval cells positive to cytokeratin 7 immunostaining  
B. apoptosis (arrow) seen in cytokeratin 7 stain  
C. HCC with pelioid pattern, intratrabecular hemorrhage (arrow). H and E  
D. the giant tumor cell with hyaline globules, H and E x 400
seen together with prominently dilated sinusoidal spaces in the pelioid pattern (Fig. 4C) [8]. Within the trabeculae of HCC, sporadic giant tumor cells each containing a large multilobated nucleus and hyaline globules in the cytoplasm were seen lying adjacent to the HCC cells (Fig. 4D). Rupturing of these cells and the release of cytoplasmic content and hyaline globules into the space of Disse was observed. The mesenchymal transformation of these giant tumor cells could be detected by the expression of alpha-actin and vimentin (Table 1).

The giant cell with a centrally placed multilobated nucleus and scant cytoplasm could be seen adjacent to the HCC cells (Fig. 5A). Sequential transformations of the polyhedral HCC cells to elongated HCC cells and actin and thereby to spindle mesenchymal cells could be traced (Fig. 5B). Large foci of hemorrhages, tumor necrosis, and inflammation represented by neutrophil lymphocyte and macrophage infiltrations were observed in the inner part of the specimen (Fig. 5C). Within the zone of spindle mesenchymal cells, sporadic giant mesenchymal cells containing hyaline globules were seen in fibrous stroma (Fig. 6A). Both spindle and giant mesenchymal cells disclosed expressions of alpha-actin and vimentin (Fig. 6B) but not of cytokeratin as summarized in Table 1. In some foci, the spindle cells formed fibrous stroma detected by Masson’s trichrome stain interpreted as fibrosarcoma (Fig. 6C).

The patient had an uneventful post operative course and after being discharged, he became lost to follow up.

**Discussion**

According to the authors’ recent study, hepatocarcinogenesis in Thailand is related to chronic
viral hepatitis B or C, aflatoxin B1 nitrosamine(s) and occasionally to some certain nodular hepatocellular lesions secondary to arterial hyperperfusion\(^{(16,22)}\). The incidence rate of HCC in Thailand is 9.7-10.7/10^5/year\(^{(23)}\). No sarcomatous transformation of the carcinoma was described in any HCC studies from different countries referred to in the authors’ recent review\(^{(19)}\). HCC-CGC is presently considered as a variant of HCC. It is estimated that it occurs in one-half of the cases of primary hepatocellular tumor diagnosed from surgical hepatic resections averaging 22 cases per year in Ramathibodi Hospital\(^{(8,24)}\).

The sarcomatous transformation of HCC, CGC, and HCC-CGC has frequently been reported from Japan since 1984\(^{(4)}\) and from Korea in 2004\(^{(17,18)}\). Recognition of the sarcomatous transformation in Thailand in 2005 may reflect a recent change in dietary habit in accordance to the present situation of urgent life style. Consumption of packaged or fast foods in place of regular home-cooked dishes of rice fish meat and vegetables is commonly practiced. This may lead to the possible exposures to high concentration of food preservative(s) i.e. nitrate and nitrite allowing the occurrence of nitrosamine(s), potent chemical carcinogen(s), in gastric acid environment\(^{(25)}\). Transformation of the epithelial cells of HCC and HCC-CGC to mesenchymal cells due to dedifferentiation was proposed by a previous report\(^{(6)}\). This viewpoint is supported by the direct transitional change of HCC cells to spindle mesenchymal cells as illustrated in Fig. 5B and considerably stronger oncofetal expression detected by glutathione S transferase-pi staining (Table 1) than HCC and HCC-CGC without sarcomatous transformation observed in the authors’ previous study\(^{(19)}\).

The present findings of extensive tumor-cell necrosis and severe inflammatory response may have lead to the fever in the presented patient. Hyperpyrexia and leukocytosis can occur in some HCC patients\(^{(26)}\). Extensive necrosis and arterial hypoperfusion in the central tumor part can be seen in the CT as hypodense lesion with the clinical impression of liver abscess\(^{(27)}\). The intratumor septation seen by CT and MRI (Fig. 1) is beneficial for the interpretation of primary liver carcinoma. The septation detected by CT and MRI may be secondary to low-signal zones due to tumor-cell necroses at the periphery of each of the multiple lobules of active tumors growth within the mother tumor in relation to the arterial blood supply. Intrahepatic non-septate cystic lesion(s) in association with peripheral blood eosinophilia is favorable for the diagnosis of the prolonged phase of hepatic fascioliasis\(^{(28)}\). Alpha-fetoprotein level may be helpful in supporting the diagnosis of primary hepatocellular carcinoma in the case of hypodense lesion\(^{(8)}\).

The presence of binucleated small HCC cells in the telophase of mitosis and oval cells in the tumor periphery may indicate a prominently rapid tumor growth at the periphery and the capability of tumor in maintaining the clonal cells of these two cell types.

In conclusion, sarcomatous transformation to various mesenchymal components in HCC, CGC, and HCC-CGC is increasingly reported mainly from Asian countries. The recent change in dietary pattern to consumption of packaged or fast foods containing food preservative(s) acting also as carcinogenic precursor(s) is here suggested for the underlying mechanism. The sarcomatous transformation may slightly alter the already poor clinical prognosis known in the patients having HCC and HCC-CGC\(^{(19,26-31)}\). This transformation should be fully aware of in the field of diagnostic pathology and should be readily differentiated from undifferentiated sarcoma of the liver occurring in pediatric patients between 5 and 15 years of age\(^{(32)}\).

References
มะเร็งปฐมภูมิของเซลล์ตับร่วมกับเซลล์ท่อน้ำดีที่มีการกลายสภาพเป็นซาร์โคมาได้ถูกวินิจฉัยเป็นครั้งแรกในโรงพยาบาลรามาธิบดีในปี พ.ศ. 2548 มะเร็งตับชนิดนี้ได้ถูกรายงานจากประเทศในทวีปเอเชียในระยะที่ไม่นานมานี้การกลายสภาพเป็นซาร์โคมาของเซลล์มะเร็งที่เกิดจากเซลล์ตับน่าจะเป็นขบวนการที่เกี่ยวข้องกับการเกิดมะเร็งตับในประเทศไทยรวมถึงโรคตับอักเสบเรื้อรัง บี หรือ ซี สารอาฟลาทอกซิน บี ไนโตรซามิน และ nodular hepatocellular lesion ที่เกิดจากการมาเลี้ยงของเลือดแดงเพิ่มมากกว่าปกติ คณะผู้รายงานคาดว่าการเปลี่ยนแปลงวิถีชีวิตที่เร่งรีบอาจทำให้ร่างกายได้รับสารเคมีที่ก่อการกลายสภาพเป็นซาร์โคมาของมะเร็งปฐมภูมิของตับได้เกิดจากเซลล์ตับอย่างเดียวหรือร่วมกับเซลล์ท่อน้ำดี