Pathogenesis of Cholangiolocellular Carcinoma: Possibility of an Interlobular Duct Origin

Fukuo Kondo \(^1\,^2\) and Toshio Fukusato \(^2\)

Abstract

Cholangiolocellular carcinoma (CoCC) is categorized as a different entity from ordinary intrahepatic cholangiocarcinoma (ICC) due to its unique clinical, radiological and histological features. The lesion is supposed to originate from cholangioles, where hepatic stem/progenitor cells exist. However, the interlobular duct is also speculated to be the origin of CoCC. According to the findings of morphometric and immunohistochemical studies, CoCC closely resembles the interlobular duct. The unique clinical and pathological features of this disease can also be explained by the interlobular duct origin theory. The malignant counterparts of cholangioles and interlobular ducts have been categorized as CoCC to date. In order to differentiate between true CoCC (cholangiole origin) and pseudo-CoCC (interlobular duct origin), assessing the size of the cancer duct, positivity for c-Kit and coexistence of an ordinary ICC component is useful.

Key words: cholangiolocellular carcinoma, interlobular duct carcinoma, intrahepatic cholangiocarcinoma, stem/progenitor cell


Introduction

Cholangiolocellular carcinoma (CoCC) consists of very thin cancer ducts and is immunohistochemically positive for various stem/progenitor cell markers (1-7). Immunohistochemistry for epithelial membrane antigens (EMAs) shows a membranous pattern (positive staining in the luminal membrane), whereas ordinary intrahepatic cholangiocarcinoma (ICC) displays a cytoplasmic pattern (positive staining in the cytoplasm) (8). The clinical features of CoCC also differ from those of ICC. For example, the imaging and macroscopic findings usually show mass formation without dilatation of the peripheral bile ducts, and the lesion is frequently associated with chronic liver disease (9-11). Due to these unique pathological and clinical features, CoCC has recently been classified as a different entity from ICC (5, 6).

However, the origin of CoCC remains controversial. Since Steiner et al. published the first report on CoCC, the interlobular duct has been speculated to be another possible origin (1). If every part of the bile duct carries the potential for malignant transformation, the interlobular duct must have a malignant counterpart. Recent morphometric and immunohistochemical findings have shown that CoCC resembles the interlobular duct more so than the cholangiole (12).

In this article, we describe the characteristics of CoCC and problems associated with its definition. In addition, we propose criteria for differentiating between true CoCC (cholangiole origin) and pseudo-CoCC (interlobular duct origin).

Characteristics of CoCC

Classification of intrahepatic bile ducts

Before describing the characteristics of CoCC, it is necessary to review the classification of intrahepatic bile ducts (13, 14) (Fig. 1). Intrahepatic bile ducts are precisely classified according to their location and size.

Cholangioles (canals of Hering) are small ducts located in peripheral areas (slightly outside) of portal tracts, without accompanying portal veins or arteries (black arrows in Fig. 1a, b). These lesions are usually smaller than 15 μm. Interlobular ducts (ILDs) and septal ducts are ducts located in the central area of the portal tract accompanying...
Figure 1. Comparison of non-neoplastic small bile ducts and CoCC (immunohistochemistry for CK7). The thin black arrows in (a) and (b) show cholangioles. The blue arrow in (a) shows an interlobular duct of small size (ILD-S). The blue arrow in (b) shows an interlobular duct of medium size (ILD-M). (c) shows a septal duct. (d) shows cancer ducts of CoCC. Bar: 50 μm (a-d). In these figures at the same magnification (a-d), the sizes of the ILD-S, ILD-M and CoCC ducts are apparently larger than that of the cholangiole. However, these ducts are clearly smaller than the septal duct. (e) CoCC on a low-magnification view. Bar: 200 μm. The duct size of CoCC appears to be very thin on this low-magnification view.

Histological features of cholangiolocellular carcinoma

The histological features of CoCC are shown in Figs. 1 and 2. In comparison with septal ducts, cancer ducts are apparently small for CoCC (Fig. 1c, d). When CoCC ducts are observed on a low-magnification view, they appear to be very thin (Fig. 1e). CoCC ducts are also smaller than those of ICC (Fig. 2a, b). Immunohistochemistry for EMA also shows a distinct difference between ICC and CoCC (Fig. 2c, d); the former exhibits positivity in the cytoplasm (cytoplasmic pattern), while the latter demonstrates positive staining in the membranous area of the lumen (membranous pattern) (8).

Clinical and imaging findings of cholangiolocellular carcinoma

Fig. 3 shows a comparison of the clinical and imaging findings of CoCC and ICC. Ordinary ICC usually exhibits dilatation of the peripheral bile ducts. The macroscopic classification can be the intraductal growth type, periductal infiltration type or mass forming type. The lesion is rarely accompanied by chronic liver disease. In contrast, CoCC lacks dilatation of the bile ducts, while the macroscopic features usually correspond to the mass forming type (9-11) and chronic liver disease is frequently associated with CoCC. These differences can be explained by the difference in tumor origin.

Due to these unique histological and clinical features, CoCC is now categorized as a different entity from ICC (5, 6).
Speculation on the origin of cholangiolocellular carcinoma

As mentioned above, CoCC and ICC show distinct differences in both clinical and pathological features. This differences are explained by the difference in tumor origin. ICC is believed to originate from ordinary intrahepatic bile ducts (e.g. septal duct or thicker intrahepatic bile ducts), whereas CoCC is thought to originate from very thin intrahepatic bile ducts (cholangiole or interlobular duct). The possibility of a cholangiole origin has recently increasingly become strongly emphasized.

Moreover, recent advances in hepatic stem/progenitor cell studies have provided evidence strengthening the cholangiole origin theory (15-19). Cholangioles are now considered to have the characteristics of stem/progenitor cells, and CoCC is speculated to be the malignant counterpart of these stem/progenitor cells. Because stem/progenitor cells can develop into both hepatocytes and cholangiocytes, they are thought to be the cause of combined hepatocellular-cholangiocarcinoma, and CoCC is categorized as a subtype of combined hepatocellular-cholangiocarcinoma of the subtype with stem cell features (6).

Problems with the Cholangiole Origin Theory

The cholangiole origin theory is very reasonable based on the characteristics of CoCC. Unfortunately, however, it has some problems, as listed below. We identified these problems during the process of diagnosing CoCC in routine practice.

Size of CoCC ducts

As shown in Fig. 1, the size of CoCC ducts is far larger than that of cholangioles. This corresponds to the intermediate size of ILD-Ss and ILD-Ms.

Staining pattern of EMA

A membranous pattern of EMA immunostaining is found in ILD-Ss and ILD-Ms as well as cholangioles (Fig. 4).

Figure 2. Comparison of ICC and CoCC based on the microscopic findings. (a) and (b) show Hema-toxylin and Eosin staining for ICC and CoCC. Bar: 500 μm. The cancer ducts of ICC (a) are clearly larger than those of CoCC (b). The inset of (b) shows an antler-like adenocarcinoma area with marked fibrous stroma of the same lesion. The cancer ducts are definitely smaller than those of ICC (a). (c) and (d) show immunohistochemistry for EMA in the ICC and CoCC ducts. Bar: 50 μm. The ICC ducts show positive staining in the cytoplasm (cytoplasmic pattern), while the CoCC ducts show positivity in the membranous area of the lumen (membranous pattern).
Specificity of stem/progenitor cell markers

Some stem/progenitor cell markers, namely, c-Kit, CD56 and EpCAM, are also positively stained in ILDs and septal ducts (Fig. 5). Although ILDs and septal ducts are not thought to have stem/progenitor cell characteristics, positivity for these stem/progenitor cell markers is not rare.

Unique clinical and imaging features

The unique clinical and imaging features of CoCC are also well explained by the ILD origin theory. Because ILD is the smallest bile duct, except for the cholangiole and bile canaliculus, carcinoma of ILD origin will not cause dilatation of the peripheral bile duct. For the same reason, the carcinoma may present as the mass forming type on images. If chronic inflammation of the peripheral portal tracts promotes the carcinogenesis of cholangiocytes of ILDs, the coexistence of CoCC and chronic liver disease is reasonable (12).

These issues reduce support for the cholangiole origin theory and suggest another possibility: the ILD origin theory. This theory has already been described by Steiner et al. (1).
In order to address the above issues, we performed morphometric and immunohistochemical studies (12). In particular, we measured the size of CoCC ducts compared with that of non-neoplastic cholangioles, ILDs and septal ducts. Two hepatocyte markers (Hep Par 1 and α-fetoprotein (AFP), two cholangiocyte markers (cytokeratin CK7 and CK19), a marker for mucin (Muc1), a hepatic stem/progenitor cell marker (c-Kit) and EMA were used for the immunohistochemical study.

**Size of CoCC ducts**

Consequently, the outer diameter of CoCC was far larger than that of the cholangiole (p<0.0001) (Fig. 6).

**Morphometric and Immunohistochemical Studies of Cholangiolocellular Carcinoma and Non-neoplastic Bile Ducts**

Figs. 1a, b and d also clearly show the difference. The size of the CoCC ducts was also significantly larger than that of ILD-Ss (p<0.0001) but significantly smaller than that of ILD-Ms (p<0.0001) (Fig. 1a, b, d, 6) and far smaller than that of the septal ducts (p<0.0001) (Fig. 1c, d, 6). The inner diameter of CoCC also showed the same tendency. These morphometric results indicate that CoCC morphologically resembles ILD more so than cholangioles.

**Immunohistochemistry**

**Hepatocyte markers:**

Both Hep Par 1 and AFP were negatively stained in all ducts of CoCC and the control ducts (Table 1). The CoCC and control ducts did not show any hepatocytic characteristics.

**Cholangiocyte markers:**

The cholangiocyte marker CK7 was positively stained in all ducts of CoCC and the control ducts. CK19 was positively stained in most of the CoCC ducts and control ducts (Table 1).

The results for CK7 and CK19 showed that CoCC and control ducts have cholangiocyte characteristics.

As far as these results are concerned, CoCC did not exhibit bipotentiality (hepatocytes and cholangiocytes).

**Marker for mucin:**

The rate of positivity for Muc1 in the CoCC ducts was significantly lower than in the control duct group (p<0.05). Although the rate of positivity was low, some CoCCs stained positively for Muc1. This result implies that CoCC is a subtype of adenocarcinoma.

**Stem/progenitor cell marker:**

c-Kit was positively stained in 1.9% of the CoCC ducts (Table 1). In contrast, the cholangioles, ILD-Ss, ILD-Ms and septal ducts also showed rates of positivity of 47.0%, 13.6%, 7.8% and 4.9%, respectively. Among the control ducts, the rate of positivity in the cholangioles was furthest from that observed in CoCC. In addition, the findings of positivity in the ILDs and septal ducts revealed that c-Kit is
not a reliable specific stem/progenitor cell marker (Fig. 5a).

**Epithelial membrane antigen:**

EMA was positively stained in almost all of the CoCC ducts and control ducts. However, the staining pattern (membranous or cytoplasmic pattern) differed depending on the type of duct (Table 1, Fig. 4). The CoCC ducts, cholangioles and ILD-Ss showed rates of positivity for a membranous pattern of nearly or precisely 100%. In addition, the staining patterns for EMA in the control ducts showed a close relationship with the duct size (outer diameter) (Fig. 7); however, this relationship differed from that presumed for a long time (8). The boundary of the membranous and cytoplasmic patterns was not the boundary of cholangioles and ILDs (15 μm). The ILD-S and ILD-M ducts also showed a membranous pattern (Fig. 4b, c); thus, the membranous pattern was not specific for cholangioles and CoCC. The true boundary showed a pattern of a transitional zone of 30 to 50 μm, with a conversion point of 40 μm. These immunohistochemical results also indicate that the characteristics of CoCC resemble those of ILDs rather than cholangioles.

**Cell size of carcinoma of the hepatic hilus and non-neoplastic cells**

In order to emphasize the importance of morphometric

---

**Table 1. Immunohistochemistry for CoCC and Various Non-neoplastic Small Bile Ducts.**

<table>
<thead>
<tr>
<th></th>
<th>Hep Par1</th>
<th>AFP</th>
<th>CK7</th>
<th>CK19</th>
<th>MUC1</th>
<th>c-Kit</th>
<th>EMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCC (n=1500)</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>89.10%</td>
<td>6.90%</td>
<td>1.90%</td>
<td>98.70%</td>
</tr>
<tr>
<td>Colangiole (n=321)</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>96.0% **</td>
<td>21.2% *</td>
<td>47.0% *</td>
<td>100%</td>
</tr>
<tr>
<td>ILD-S (n=382)</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>94.2% **</td>
<td>30.4% *</td>
<td>13.6% *</td>
<td>100%</td>
</tr>
<tr>
<td>ILD-M (n=180)</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>97.2% **</td>
<td>18.3% *</td>
<td>7.8% *</td>
<td>100%</td>
</tr>
<tr>
<td>Septal duct (n=82)</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>97.6% **</td>
<td>25.6% *</td>
<td>4.90%</td>
<td>100%</td>
</tr>
</tbody>
</table>

CoCC: cholangiolocellular carcinoma, ILD-S: interlobular duct (small size), ILD-M: interlobular duct (medium size)

M: membranous pattern, C: cytoplasmic pattern

versus cholangiolocellular carcinoma

p<0.0001, ** p<0.05

Adapted from Maeno et al. (12).
data, we present the morphometric data for a comparison of carcinoma of the hepatic hilus and non-neoplastic cells (Fig. 8, 9). Some pathologists have claimed that morphometric data are not useful because carcinoma cells increase in
Table 2. Malignant Counterparts of the Various Non-neoplastic Bile Ducts: Comparison of the Present and New Concepts.

<table>
<thead>
<tr>
<th></th>
<th>Present concept</th>
<th>New concept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholangioles</td>
<td>CoCC</td>
<td>True CoCC (ICC of ductular type)</td>
</tr>
<tr>
<td>Interlobular ducts</td>
<td>???</td>
<td>Pseudo-CoCC (Interlobular duct carcinoma, or ICC of small duct type)</td>
</tr>
<tr>
<td>Septal ducts</td>
<td>ICC</td>
<td>ICC</td>
</tr>
<tr>
<td>Large ducts</td>
<td>ICC</td>
<td>ICC</td>
</tr>
</tbody>
</table>

Table 3. Differentiation of True Cholangiocellular Carcinoma and Pseudo-cholangiocellular Carcinoma (Interlobular Duct Carcinoma).

<table>
<thead>
<tr>
<th></th>
<th>True CoCC (ICC of ductular type)</th>
<th>Pseudo-CoCC (Interlobular duct carcinoma, or ICC of small duct type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Useful</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of duct</td>
<td>&lt;15μm</td>
<td>15~100μm</td>
</tr>
<tr>
<td>c-Kit</td>
<td>High positive ratio</td>
<td>Low positive ratio</td>
</tr>
<tr>
<td>Co-existence of ICC</td>
<td>(-)</td>
<td>Frequent</td>
</tr>
<tr>
<td>Not Useful</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMA</td>
<td>Membranous</td>
<td>Membranous</td>
</tr>
<tr>
<td>Hep Par 1</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>CK7</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>CK19</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Mucin</td>
<td>(-)~Low positive ratio</td>
<td>(-)~Low positive ratio</td>
</tr>
</tbody>
</table>

Non-neoplastic Bile Ducts and Their Malignant Counterparts

If every part of the bile duct shows malignant transformation, every part must have a malignant counterpart. At present, however, the malignant counterpart of ILDs (interlobular duct carcinoma) is lacking (Table 2). Interlobular duct carcinoma is categorized as CoCC together with true CoCC. It is thus necessary to establish a new concept in which interlobular duct carcinoma (pseudo-CoCC) and true CoCC are clearly differentiated (Table 2). In Tables 2 and 3, the terms ICC of small duct type and ICC of ductular type were used because they are good alternatives for pseudo-CoCC and true CoCC. In the future, the terminology may be revised. At present, however, the term CoCC is very useful for referring to this unique primary adenocarcinoma of the liver.

We propose the following criteria for differentiating true and pseudo-CoCC. Table 3 lists various items used to diagnose CoCC. Among these, the expressions of EMA, Hep Par 1, CK7, CK19 and mucin are not very useful because...
the non-neoplastic counterparts show the same features (Table 1). In contrast, the duct size and rate of positivity for c-Kit are thought to be useful based on the findings of a previous study (Table 1) (12). The coexistence of an ordinary ICC component is also a helpful finding. Ordinary ICC components with a cytoplasmic EMA pattern frequently coexist with CoCC components (12). Because a cytoplasmic EMA pattern is a feature of ILD-Ms, septal ducts and larger bile ducts, the coexistence of features of ILDs and larger ducts is more likely than the coexistence of features of cholangioles and larger ducts. Accordingly, a considerable number of CoCC lesions must be reclassified as interlobular duct carcinoma.

In fact, all 15 CoCC lesions assessed in our former study (12) were reclassified as the pseudo-CoCC (interlobular duct carcinoma) dominant type according to the criteria shown in Table 3. In each case, most of the cancer ducts exhibited a size of pseudo-CoCC (15-100 μm). Only nine of 1,500 cancer ducts displayed a size of true CoCC (smaller than 15 μm). The true CoCC type cancer ducts and pseudo-CoCC type cancer ducts showed a similarly high incidence of a membranous EMA pattern and low incidence of c-Kit positivity. These immunohistochemical results suggest that the 15 CoCCs were almost all pure pseudo-CoCC lesions.

**Histological Heterogeneity of CoCC**

In addition to the above 15 homogeneous type CoCCs, primary adenocarcinoma of the liver frequently demonstrates the coexistence of ICC and CoCC components. Well, moderately and poorly differentiated ICC and CoCC components sometimes coexist. These histological patterns may reflect progressive features of ICC or CoCC. Even under such conditions, the EMA immunohistochemical pattern is useful for classifying ICC and CoCC components. The former component usually shows a cytoplasmic pattern, while the latter demonstrates a membranous pattern. Furthermore, HCC components also coexist with ICC and CoCC components (6). These lesions are interpreted to be mixed or combined tumors of hepatocellular cholangiocarcinoma.

**CoCC Component in Combined Hepatocellular Cholangiocarcinoma**

Finally, the characteristics of CoCC components coexisting with HCC should be discussed. Fig. 10 shows the histological features and immunostaining patterns of the CoCC component of combined HCC-CC with stem cell features, cholangiolocellular type. The CoCC component is visualized as a smaller area (6 mm in diameter) within the larger background HCC nodule (16 mm in diameter). The histological and immunohistochemical features closely resemble those of CoCC of the pure adenocarcinoma type, as shown in Figs. 1d, e and 2b, d. With respect to the morphological and immunohistochemical features, there are no differences between the two CoCC components.

As to the origin of these two types of CoCC components, it is necessary to describe the differences. The pure adenocarcinoma type is believed to originate from the interlobular duct, whereas the combined carcinoma type is formed by the transdifferentiation of the background HCC tissue, as the CoCC component in Fig. 10 was found to present as a smaller area within the larger background HCC area. The transdifferentiation of HCC may be another pathway causing so-called CoCC. This transdifferentiation pathway should be studied more intensely in the future.

**Conclusion**

Based on the results of previous excellent studies, the classification of CoCC and mixed hepatocellular-cholangiocarcinoma has greatly advanced (5, 6). Although we greatly admire these previous works, they are not entirely perfect, and it is therefore necessary to advance the classification further.

This article places emphasis on the possibility of an interlobular duct origin based on the findings of our former study (12). The concept of interlobular duct carcinoma, namely the malignant counterpart of ILDs, must be established. In addition, these lesions should be clearly differentiated from true CoCC of cholangiole origin, and the mechanism underlying the formation of the CoCC component within combined HCC-CC lesions should be clarified in the future.

**The authors state that they have no Conflict of Interest (COI).**

**Financial Support**

This article (Fukuo Kondo) was supported in part by the grants from The Vehicle Racing Commemorative Foundation.

**References**


© 2015 The Japanese Society of Internal Medicine
http://www.naika.or.jp/imonline/index.html