Primary Liver Carcinoma of ‘Intermediate’ (Hepatocyte-Biliary Epithelial Cell) Phenotype

Thesis by

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Primary Liver Carcinoma of ‘Intermediate’ (Hepatocyte-Biliary Epithelial Cell) Phenotype

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There is increasing evidence of 'oval-like' hepatic progenitor cells in human liver disease. It is a matter of debate whether primary liver carcinoma with 'intermediate' (hepatocyte-biliary epithelial cell) phenotype arises from transformed progenitor cells with the bipotential to differentiate into hepatocytes and biliary epithelial cells. In this study, primary liver carcinomas with intermediate features were identified and an immunohistochemical analysis was performed using hepatocytic markers (alpha-fetoprotein and hepatocyte), biliary epithelial cell markers (carcinoembryonic antigen and cytokeratin 19) and progenitor cell marker (*c-kit*), in order to study the origin of primary 'intermediate' carcinomas.

Forty-seven cases of primary liver carcinomas were the subject of study, and ten of these cases demonstrated strands or trabeculae of small, uniform, round to oval cells with scanty cytoplasm and hyperchromatic nuclei embedded within a thick desmoplastic stroma, and hence named 'intermediate' (hepatocyte-biliary epithelial cell) carcinomas. Four cases were designated transitional type CHC. Nine were named HCC, small cell type, demonstrating morphological features almost compatible with typical HCCs except that they were composed of smaller

cells. The remaining cases consisted of 20 typical HCCs and 4 typical CCs. Six of 10 ‘intermediate’ carcinomas showed simultaneous expression of hepatocytic (AFP or hepatocyte) and biliary (CEA or CK19) markers and six showed positive immunohistochemistry for *c-kit*. Of the 4 cases of transitional CHCs, two expressed both hepatocytic and biliary markers, and one of these 2 cases was positive for *c-kit*. Three cases of HCC, small cell type were positive for both hepatocytic and biliary markers but none expressed *c-kit* and the other 6 cases demonstrated expression of *c-kit* but were negative for biliary markers. The 20 typical HCCs expressed only hepatocytic markers and the 4 typical CCs expressed only biliary markers.

From these results it can be suggested that ‘intermediate’ (hepatocyte-biliary epithelial cell) carcinoma is a distinct type of primary liver carcinoma originating from transformed hepatic progenitor cells that have the bipotential to differentiate into both hepatocytic and biliary lineage.

Key Words: Hepatocellular carcinoma, cholangiocarcinoma, combined hepatocellular-cholangiocarcinoma, *c-kit*, progenitor cell, intermediate phenotype

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INTRODUCTION

Primary liver carcinomas have been classified into hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) originating from hepatocytes or hepatocyte precursor cells and biliary epithelial cells, respectively. However, there have been a few reported cases in the literature of combined hepatocellular-cholangiocarcinomas (CHC)[1-4], a subset of primary liver carcinomas suggested to arise from transformed 'oval-like' progenitor cells which have the bipotential to differentiate into both hepatocytes and biliary epithelial cells. Taguchi et al.[3] classified CHC into three types, (i) type I, in which there were clearly separable areas of HCC and CC, (ii) type II, in which the HCC and CC areas were contiguous with an intervening area of transition, and (iii) type III, in which the tumor was not readily classifiable as HCC or CC, and were composed of tumor cells showing morphological features intermediate between HCC and CC. Robrechts et al.[5] reported a case of 'liver tumor of intermediate (hepatocyte-bile duct cell) phenotype', consisting of small cells with a phenotype intermediate between hepatocytes and biliary epithelial cells and simultaneously expressing cytokeratins 7, 8, 18 and 19. These tumors consisted of relatively small and monomorphous cells with a

small rim of cytoplasm and hyperchromatic nucleus, arranged in strands and trabeculae and surrounded by delicate fibrous stroma. Wu et al.[6,7] conducted immunohistochemical analysis of hepatocellular carcinomas with dual (hepatocellular/biliary) phenotypes in Chinese patients, and found that the tumors displaying simultaneous expression of cytokeratin 19, AE1/AE3 and HepPar 1 also expressed CK 14, a progenitor cell marker. It has therefore been postulated that these carcinomas are either derived from 'oval-like' progenitor cells, or have undergone dedifferentiation to the bipotential progenitor cell phenotype during carcinogenesis.

For a long time there has been active research on hepatic stem cells in experimental animals. After inciting injury by using different means in experimental rats, isolated or scattered groups of non-parenchymal oval-shaped epithelial cells, which expressed OV-6, appeared around portal tracts prior to hepatocyte regeneration or bile duct cell proliferation, and owing to their morphological features, they were named oval cells [8]. These oval cells were found to function as a stem cell compartment, activated when proliferation of hepatocytes was not feasible. Morphological evidence of the existence of the human counterpart of these oval cells, also named 'intermediate' cells by some authors, was reported recently [9-11]. They were best seen either isolated or in linear configurations in regenerative nodules around hepatocellular carcinomas, in chronic cholestatic disease, focal nodular hyperplasia, hepatoblastoma, cirrhotic liver and the developing human liver[12-16], and from experimental evidence that they co-express cytokeratin 7, 19, alpha-fetoprotein (AFP) and OV-6, it was suggested that they may act as progenitor cells with the potential of both hepatocytic and biliary differentiation.

During the past three years, there has been increasing interest in the concept that bone marrow-derived hematopoietic stem cells can differentiate into

hepatic epithelium [17-24]. *C-kit* is a proto-oncogene encoding a transmembrane tyrosine kinase receptor (CD117) that is structurally similar to the receptors for platelet-derived growth factor (PDGF) and colony-stimulating factor-1 (CSF-1), and is expressed in mast cells and hematopoietic progenitor cells, playing an essential role in hematopoiesis and proliferation and/or migration of primordial germ cells and melanoblasts during embryogenesis. Canals of Hering and proliferating progenitor cells in diseased human liver were reported to express *c-kit*[25], and a proportion of these hepatic progenitor cells are considered to originate from bone marrow-derived hematopoietic stem cells.

It can be hypothesized that primary liver carcinomas with 'intermediate' (hepatocyte-biliary epithelial cell) phenotype may express *c-kit*, in addition to simultaneous expression of hepatocytic and biliary markers. In this study, an immunohistochemical analysis of primary liver carcinomas with ‘intermediate’ phenotype was performed, using primary antibodies to alpha-fetoprotein (AFP), hepatocyte, carcinoembryonic antigen (CEA), CK 7, CK 19 and *c-kit*, to find out whether these tumors are of bipotential hepatic progenitor cell origin.

MATERIALS AND METHODS

1. Materials

Among 185 consecutive cases of primary liver cancers surgically resected at Yonsei University College of Medicine between February 2000 and August 2002, 47 cases were selected after histological examination of routine hematoxylin-eosin stained sections.

The 47 cases included (i) ‘intermediate’ (hepatocyte-biliary epithelial cell) carcinomas: tumors with intermediate differentiation, showing small, monomorphous cells with a small rim of cytoplasm and hyperchromatic nuclei, forming strands and trabeculae, and embedded in a background of desmoplastic stroma, (ii) transitional CHCs: tumors showing apparent transition between HCC and CC, with solid nests of hepatoid cells and focal glandular architecture separated by broad desmoplastic stroma, and (iii) HCC, small cell type: tumors with morphological features of HCC but predominantly composed of smaller cells arranged in a trabecular pattern.

Twenty cases of typical HCC and four cases of typical CC were also included as controls: HCCs were characterized by tumors cells with abundant eosinophilic cytoplasm, large vesicular nuclei and prominent nucleoli, arranged in a trabecular growth pattern and lack of significant desmoplasia, whereas CCs were tumors with significant desmoplasia, prominent glandular formations lined by cuboidal cells with or without mucin production.

The clinical data were obtained from hospital charts and tumor registry files in each case, and included age, sex, tumor size, serological markers for hepatitis B virus (HBV) and hepatitis C virus (HCV) and history of alcohol intake.

2. Immunohistochemistry

Four-μm paraffin tissue sections were cut from best preserved representative paraffin blocks for immunohistochemical staining. The paraffin sections were deparaffinized in xylene for 15 minutes, and rehydrated in 100%, 90% and 70% alcohol for 1 minute each. After treatment with 3% hydrogen peroxide solution for 10 minutes to quench endogenous peroxidase, sections were boiled in 10 mmol/L citrate buffer (pH6.0) in a microwave oven for 20 minutes. Subsequently, the sections were incubated at 4℃ overnight with antibodies against alpha-fetoprotein (AFP; monoclonal, DAKO, Carpinteria, CA, USA, 1:300), hepatocyte (monoclonal, DAKO, Glostrup, Denmark, 1:50), cytokeratin 19 (monoclonal, DAKO, Glostrup, Denmark, 1:50), carcinoembryonic antigen (CEA: polyclonal, BioGenex, CA, USA, 1:200) and *c-kit* (polyclonal, DAKO, Kyoto, Japan, 1:50). After thorough rinsing in phosphate buffered saline, the sections were treated with the Histofine Simple Stain MAX PO kit (Universal Immuno-peroxidase Polymer, Anti-Mouse and -Rabbit; Nichirei, Tokyo, Japan) at room temperature for 30 minutes, and they were developed using amino-ethyl carbazole (AEC; Biomeda Corp., Foster, CA, USA), rinsed with water and counterstained with Mayer's hematoxylin.

Cytoplasmic staining in more than 5% of tumor cells was regarded as positive for AFP, hepatocyte, CK19 and *c-kit*. For CEA, tumor cells demonstrating cytoplasmic expression were counted as positive, and those with canalicular expression were regarded as negative.

RESULTS

1. Clinical Features

The clinical features are tabulated in table 1. Of the 47 selected carcinomas, ten were classified as ‘intermediate’ carcinoma, four as transitional type CHC, and nine as HCC, small cell type. The remaining twenty-four included 20 cases of typical HCC and 4 cases of typical CC.

The mean (range) patient age was 51.9 (37-68) years, 60 (50-69) years, 52.9 (41-68) years for ‘intermediate’ carcinomas, transitional type CHCs, HCCs of small cell type, respectively. The male:female ratio was 9:1, 0.33:1, and 9:1, respectively. Of the 10 ‘intermediate’ carcinomas, 6 (60%) cases were associated with hepatitis B or C viral infection, and 1 (10%) case with alcoholic hepatitis. Three (75%) out of 4 transitional type CHCs and all 9 (100%) HCCs of small cell type were associated with hepatitis B or C viral infection.

As for the typical HCCs and CCs, the mean (range) patient age was 49.7 (33-72) years and 55.3 (39-70) years, respectively, and the male:female ratio for typical HCCs was 6.7:1 whereas all patients with typical CC were male. All but one (95%) case of typical HCC were associated with hepatitis B virus infection, while none (0%) of the typical CCs showed an association with hepatitis virus infection.

2. Pathological Features

A. Histologic features

1. ‘Intermediate’ carcinoma

Ten (20.8%) of the 48 cases were composed of strands or trabeculae of small, uniform cells with scanty cytoplasm and hyperchromatic nuclei in a background of broad desmoplastic stroma, and were hence named

‘intermediate’ (hepatocyte-biliary epithelial cell) carcinoma according to their histological features (Figures 1 and 2). Mucin production was not seen. On examining the surrounding non-neoplastic liver, 8 (80%) out of 10 cases of ‘intermediate’ carcinoma showed either cirrhosis or chronic hepatitis.

2. Transitional CHC

Four (8.3%) cases showed solid nests of hepatoid cells with focal glandular architecture separated by broad desmoplastic stroma, representing areas of apparent transition between HCC and CC, and were named transitional CHCs (Figure 3). Within the transitional areas, small and uniform tumor cells with scanty cytoplasm and hyperchromatic nuclei as seen in ‘intermediate’ carcinomas were noted. Three (75%) out of 4 cases of transitional type CHCs showed cirrhosis in the surrounding liver.

3. HCCs, small cell type

Nine (18.8%) cases were predominantly composed of small neoplastic cells with slightly more abundant eosinophilic cytoplasm than in the ‘intermediate’ carcinomas but less than in typical HCCs (figure 4). Unlike typical HCCs, stromal desmoplasia was noted, but was only mild and focal compared to the tumors designated ‘intermediate’ carcinoma and transitional CHC. In all 9 cases (100%), the tumors arose in a background of cirrhosis or chronic hepatitis.

4. Typical HCCs and CCs

The 20 cases of typical HCC demonstrated tumor cells with abundant eosinophilic cytoplasm, large vesicular nuclei and prominent nucleoli, arranged in a trabecular growth pattern and without significant desmoplasia, whereas the 4 cases of CC were tumors with significant desmoplasia, prominent glandular formation, and lined by cuboidal cells with or without mucin production. All 20 cases of typical HCC showed either cirrhosis or

chronic hepatitis in the remaining liver, whereas none of the 4 typical CCs was associated with underlying cirrhosis or chronic hepatitis.

B. Immunohistochemical Findings

The immunohistochemical findings are summarized in table 1.

1. ‘Intermediate’ (hepatocyte-biliary epithelial cell) carcinoma

In 6 (60%) of the 10 ‘intermediate’ carcinomas (1, 2, 3, 4, 5 and 10), the tumor cells simultaneously expressed hepatocytic (AFP or hepatocyte) and biliary markers (CEA or CK19) and the tumor cells in 6 (60%) cases (1, 2, 5, 6, 8 and 10) also expressed *c-kit.*

2. Transitional CHC

Of the 4 cases of transitional CHCs, the tumor cells of the transitional zone expressed both hepatocytic and biliary markers in 2 (50%) cases (cases 11 and 12), and one of these 2 cases was positive for *c-kit* (case 12). The hepatocytic markers were predominantly expressed in the hepatoid areas and biliary markers were mainly expressed in the glandular areas. *c-kit* expression in case 12 was noted in the small cells within the transitional zone showing intermediate features. Only biliary marker expression was significant for the remaining 2 cases, however, focal (less than 5%) co-expression of hepatocytic markers which was insufficient to be regarded as positive in this study was actually noted in these cases.

3. HCC, small cell type

Three (33.3%) out of 9 cases of HCC with small cells were positive for hepatocytic and biliary markers (cases 15 to 18), however, none of these cases expressed *c-kit*. The remaining 6 (66.7%) cases of HCC with small cells (cases 19 to 24) showed focal expression of *c-kit* but no expression of biliary markers.

4. Typical HCCs and CCs

Of the 20 cases of typical HCC, none showed expression of biliary markers or *c-kit*. All 4 cases of typical CCs expressed biliary markers only.

Table 1. Clinical features and immunohistochemical findings of the selected primary liver carcinomas.

Case	Dx	Sex	Age	Surrounding liver	Etiologic factor	Size (cm)	AFP	Hepatocyte	CEA	CK19	*c-kit*
1	IC	M	47	LC	HBV	3	+	+	+	+	+
2	IC	M	68	CH	Alcohol	8	+	+	+	+	+
3	IC	M	56	LC	HBV	6.5	+	+	+	+	-
4	IC	M	64	CH	HCV	2.5	+	-	-	+	-
5	IC	M	54	CH	U	2	-	+	-	+	+
6	IC	F	54	Nr	U	4.5	-	-	+	+	+
7	IC	M	56	Nr	U	8.5	-	-	+	+	-
8	IC	M	40	LC	HBV	4	-	-	-	+	+
9	IC	M	37	CH	HBV	5	-	-	-	+	-
10	IC	M	43	CH	HBV	8	-	+	+	+	+
11	CHC	F	54	LC	HBV	5.5	+	+	+	+	-
12	CHC	F	50	Nr	U	6	-	+	+	+	+
13	CHC	F	67	LC	HCV	2.5	-	-	+	+	-
14	CHC	M	69	LC	HCV	2	-	-	-	+	-
15	HCCS	F	45	CH	HBV	8	+	+	-	+	-
16	HCCS	M	52	LC	HBV	4	+	+	-	+	-
17	HCCS	M	59	LC	HBV	4	+	+	-	+	-
18	HCCS	M	49	LC	HBV	4	+	+	-	-	+
19	HCCS	M	49	CH	HBV	13	+	+	-	-	+
20	HCCS	M	65	CH	HCV	2.5	+	+	-	-	+
21	HCCS	M	48	CH	HBV	15	+	+	-	-	+
22	HCCS	M	68	CH	HCV	3	-	+	-	-	+
23	HCCS	M	41	LC	HBV	5	-	-	-	-	+

(IC: ‘intermediate’ carcinoma, CHC: combined hepatocellular-cholangiocarcinoma, transitional type, HCCS: HCC, small cell type, LC: liver cirrhosis, CH: chronic hepatitis, AH: adenomatoid hyperplasia of ductal glands, PF: portal/periportal fibrosis, Nr: normal, HBV: hepatitis B virus, HCV: hepatitis C virus, DM: diabetes mellitus, CS: Clonorchis sinensis, U: unknown etiology)

Table 1 continued. Clinical features and immunohistochemical findings of the selected primary liver carcinomas.

Case	Dx	Sex	Age	Surrounding liver	Etiology	Size (cm)	AFP	Hepatocyte	CEA	CK19	*c-kit*
24	HCC	M	43	CH	HBV	15	+	+	-	-	-
25	HCC	F	56	CH	HBV	6	+	+	-	-	-
26	HCC	M	49	LC	HBV	4.5	+	+	-	-	-
27	HCC	M	54	CH	HBV	4	+	+	-	-	-
28	HCC	M	46	LC	HBV	10	+	+	-	-	-
29	HCC	F	36	CH	HBV	3.5	+	+	-	-	-
30	HCC	M	41	LC	HBV	8	-	+	-	-	-
31	HCC	M	60	LC	DM	5	-	+	-	-	-
32	HCC	M	40	LC	HBV	11	-	+	-	-	-
33	HCC	M	56	CH	HBV	16	-	+	-	-	-
34	HCC	M	61	CH	HBV	4	-	+	-	-	-
35	HCC	M	56	LC	HBV	4	-	+	-	-	-
36	HCC	M	64	CH	HBV	4.3	-	-	-	-	-
37	HCC	M	38	LC	HBV	6.5	-	-	-	-	-
38	HCC	M	33	CH	HBV	5	+	+	-	-	-
39	HCC	M	45	CH	HBV	7	-	+	-	-	-
40	HCC	F	53	CH	HBV	5	-	+	-	-	-
41	HCC	M	72	CH	HBV	3.5	-	-	-	-	-
42	HCC	M	56	LC	HBV	4.5	-	-	-	-	-
43	HCC	M	35	LC	HBV	11	-	-	-	-	-
44	CC	M	50	AH	CS	7	-	-	+	+	-
45	CC	M	39	PF	U	4	-	-	+	+	-
46	CC	M	70	PF	U	12	-	-	+	+	-
47	CC	M	62	PF	U	4	-	-	+	+	-

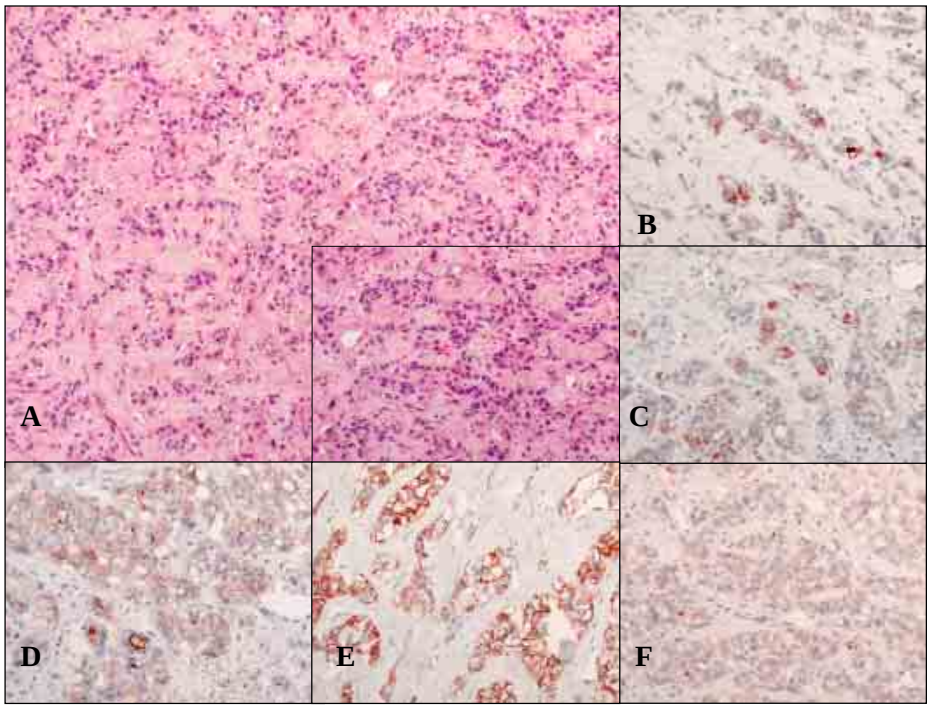

Figure 1. Histological and immunohistochemical findings of ‘intermediate’ (hepatocyte-biliary epithelial cell) carcinoma (case 1). (A) The tumor is characterized by thin strands of small round to oval cells with scanty cytoplasm and hyperchromatic nuclei, embedded within a desmoplastic stroma. Hematoxylin and eosin-stained section. x100 magnification. Immunohistochemical staining results for (B) AFP, (C) hepatocyte, (D) CEA, (E) CK19, and (F) *c-kit* are positive in the neoplastic cells. x200 magnification.

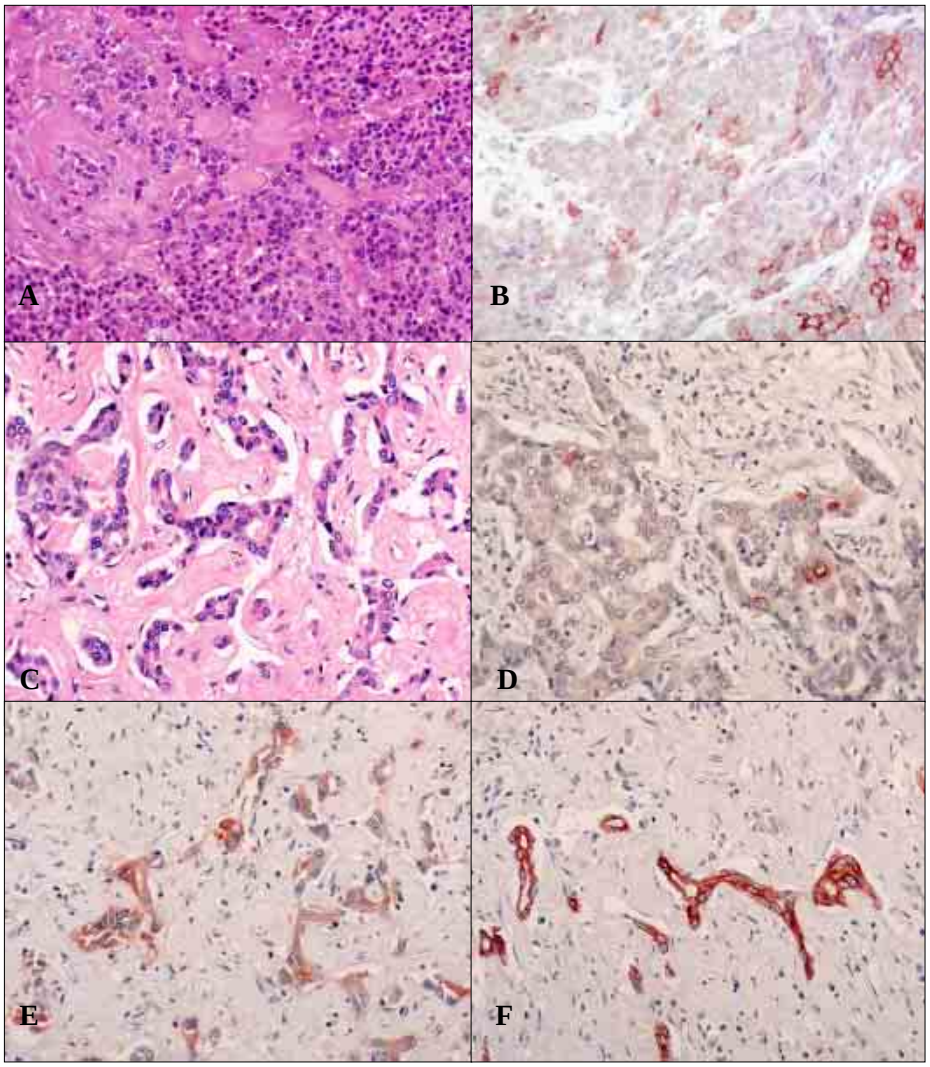

Figure 2. Histological and immunohistochemical findings of ‘intermediate’ (hepatocyte-biliary epithelial cell) carcinomas (A, B: case 6, C-F: case 3). The tumor cells of ‘intermediate’ carcinomas (A, C) are uniform in size with scanty eosinophilic to amphophilic cytoplasm and hyperchromatic nuclei. (B) *c-kit* positivity is noted in the tumor cells shown in (A). The tumor cells in case 3 (C) express both hepatocytic (D: AFP) and biliary (E: CEA, F: CK19) markers.

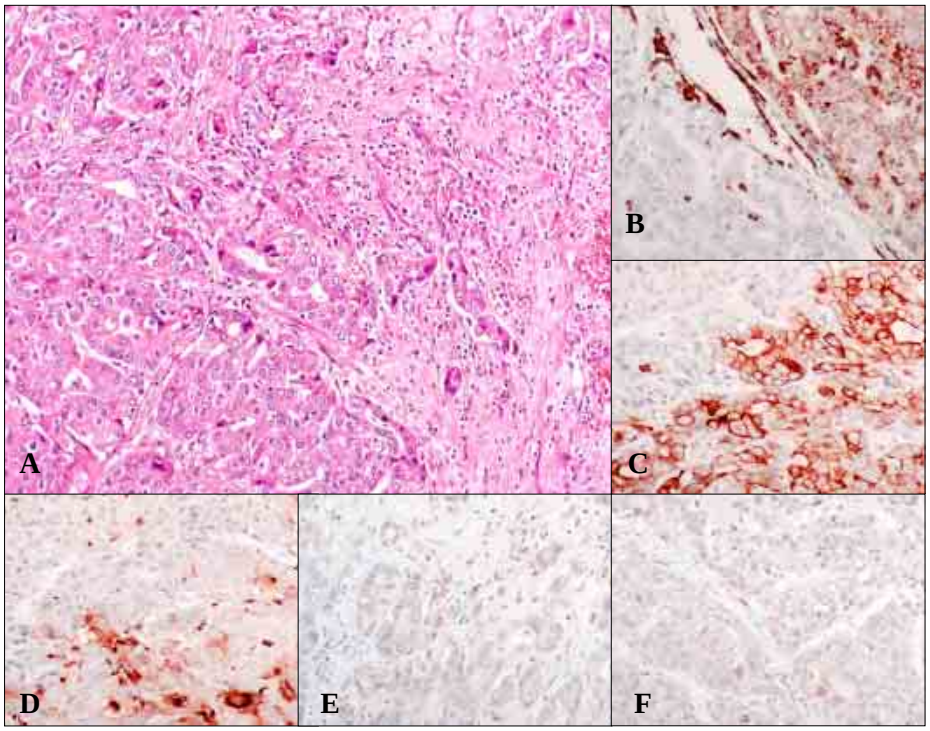

Figure 3. Histological and immunohistochemical findings of transitional combined hepatocellular-cholangiocarcinoma (case 12). (A) Small neoplastic cells with eosinophilic cytoplasm reminiscent of hepatocellular carcinoma form vague glandular structures admixed with solid hepatoid nests. The tumor cells within the hepatoid regions show expression of hepatocyte antigen (B), whereas those within the glandular areas express the biliary markers, cytokeratin 19 (C). The glandular areas demonstrate diffuse cytoplasmic expression of CEA in contrast with the adjacent hepatoid areas where CEA is only expressed in a canalicular pattern (D). (E) and (F) shows granular cytoplasmic staining of *c-kit* in the tumor cells of both the glandular and hepatoid regions.

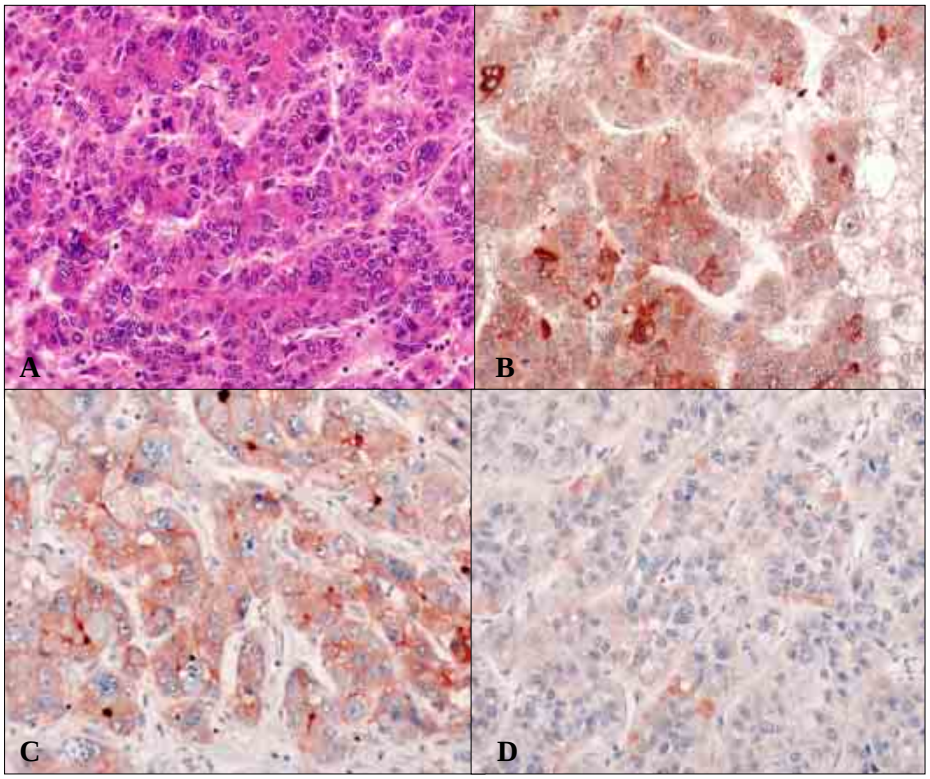

Figure 4. Histological and immunohistochemical findings of hepatocellular carcinoma of small cell type (A-C: case 20; D: case 22). Case 20 shows small neoplastic cells with eosinophilic cytoplasm, reminiscent of typical hepatocellular carcinoma, are arranged in cords set within a background of mild desmoplasia (A). They show expression of hepatocyte antigen (B), and cytoplasmic and canalicular expression of CEA (C). Case 22 also demonstrates small neoplastic hepatoid cells arranged in cords and trabeculae with slight background fibrosis, which express *c-kit* on immunohistochemical stain (D).

IV. DISCUSSION

Primary hepatic carcinomas with features intermediate between hepatocyte and biliary epithelial cells, which could be interpreted as HCC transformed to CC, or carcinoma that originated from hepatic progenitor cells that incompletely differentiated to both HCC and CC, were the main focus of this study and given the nomenclature of 'intermediate' (hepatocyte-biliary epithelial cell) carcinoma. There is increasing evidence of the presence of progenitor cells in the human liver and from the finding that canals of Hering are positive for the hematopoietic cell markers, *c-kit* and/or CD34[26, 27], it can be speculated that some hepatic progenitor cells residing in or circulating to the liver may integrate into the canals of Hering and differentiate into hepatocytes or bile duct cells.

It can be suggested that tumor cells that demonstrate features intermediate between HCC and CC cells may be the result of neoplastic transformation of hepatic progenitor cells and therefore may express *c-kit*, along with hepatocytic and biliary epithelial markers. In this study, 10 cases were designated 'intermediate' (hepatocyte-biliary epithelial cell) carcinoma from histological features. They were predominantly composed of small round to oval cells with scanty eosinophilic or amphophilic cytoplasm and hyperchromatic nuclei, forming strands or trabeculae and separated by broad desmoplastic stroma. The neoplastic cells of all 10 (100%) cases demonstrated expression of biliary epithelial markers and 6 (60%) of these cases also expressed hepatocytic markers. Six (60%) of the 10 'intermediate' (hepatocyte-biliary epithelial cell) carcinomas demonstrated expression of *c-kit*, and in four (66.7%) of these *c-kit* positive cases the same tumor cells simultaneously expressed hepatocytic and biliary epithelial cell markers. These findings are in accord with the proposal that *c-kit*-positive hepatic progenitor cells have the potential to differentiate into both hepatocytes and biliary epithelial cells and that this also applies to the neoplastic counterpart of

hepatic progenitor cells. The remaining 2 (33.3%) *c-kit*-positive cases did not show expression of hepatocytic markers, however, this may be explained by the relative insensitivity of hepatocytic markers; AFP is known to be positive in only 25-50% of HCCs by immunohistochemistry, and although hepatocyte marker has been shown to stain more than 90% of HCCs, the sensitivity decreases with poorer differentiation, and the 'intermediate' (hepatocyte-biliary epithelial cell) carcinomas in this study were in fact tumors which were too primitive to be classified as either HCC or CC[28].

The 4 cases designated transitional CHCs, corresponding to type II CHC of the classification suggested by Taguchi et al., showed small cells resembling those seen in 'intermediate' (hepatocyte-biliary epithelial cell) carcinomas within the area of transition. Two (50%) of these cases were positive for both hepatocytic and biliary epithelial markers within the transitional zone. One (25%) case demonstrated weak and focal expression of *c-kit* along with strong expression of both hepatocytic and biliary markers in the transitional area. This also may serve as evidence that transitional type CHCs originate from a bipotential progenitor cell.

Three (33.3%) cases categorized as small cell type HCC, which showed morphological features of typical HCCs but consisting of smaller cells and somewhat increased fibrous stroma traversing the tumor sheets or trabeculae, expressed both hepatocytic and biliary markers, and 6 (66.7%) out of 9 cases demonstrated focal expression of *c-kit*. This suggests that a proportion of small cell type HCCs arise from more primitive cells than typical HCCs.

The clinical significance of 'intermediate' (hepatocyte-biliary epithelial cell) carcinoma remains obscure. There have been reports that CHCs and HCC showing biliary differentiation have a more dismal overall prognosis than pure HCCs or CCs, and the primary 'intermediate' liver carcinoma described by

Robrechts et al. showed an aggressive clinical course [5]. Further study with long-term follow up will be necessary for a proper determination of the clinical significance of this subset of primary liver tumors.

V. CONCLUSIONS

In the present study, histological and immunohistochemical analysis was performed on 48 cases of primary liver carcinomas to identify ‘intermediate’ (hepatocyte-biliary epithelial cell) carcinomas.

Ten such cases were identified based on histological features and they consisted of small and uniform round to oval cells with hyperchromatic nuclei and scanty eosinophilic to amphophilic cytoplasm arranged in strands or trabeculae and embedded within thick fibrous stroma. Six ‘intermediate’ (hepatocyte-biliary epithelial cell) carcinomas simultaneously expressed hepatocytic (AFP and hepatocyte) and biliary markers (CEA and CK19), and six demonstrated expression of *c-kit*.

These results show that the primary ‘intermediate’ (hepatocyte-biliary epithelial cell) carcinomas of the liver show histological features intermediate between HCCs and CCs, and that a proportion of these tumors demonstrates expression of both hepatocytic and biliary markers along with the hematopoietic stem cell marker *c-kit*. It can therefore be concluded that primary ‘intermediate’ (hepatocyte-biliary epithelial cell) carcinoma of the liver may be a distinct type of liver carcinoma, which arises from hepatic progenitor cells with the potential of differentiation into both hepatocytic and biliary lineage.

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국문요약

원발성 '중간형' (간세포-담관상피세포) 간암종

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원발성 간암종은 일반적으로 각각 간세포나 전구 간세포, 그리고 담관상피세포에서 기원하는 간세포암종과 담관상피암종으로 분류한다. 그 외에 혼합 간세포-담관상피암종이 보고된 바 있으며, 이러한 암종의 기원세포로는 난원 모양의 전구세포의 가능성이 제시되고 있다. 이러한 난원 모양의 전구세포는 간세포와 담관상피세포로 모두 분화할 수 있는 이중분화능력을 가진 세포로 알려졌다. 본 연구에서는 간세포암종과 담관상피암종의 중간형태를 보이는 '중간형'의 원발성 간암종을 대상으로 간세포 표지자(alpha-fetoprotein, hepatocyte), 담관상피세포 표지자(carcinoembryonic antigen, cytokeratin 19) 및 전구세포(progenitor cell) 표지자(c-kit)에 대한 면역조직화학염색을 시행하여 이러한 '중간형'(간세포-담관상피세포) 간암종이 간에 존재하는 전구세포에서 기원하는 것을 밝히고자 하였다.

총 48 예의 원발성 간암종을 연구대상으로 하였으며, 이중 10 예는 '중간형'(간세포-담관상피세포) 간암종으로 치밀한 섬유조직으로 둘러싸여 있으면서 코드상 배열을 취하고 있는 작고 크기가 일정한 둥근 또는 난원형 종양세포들로 이루어져 있었으며, 종양세포들은 세포질이 적으면서 과염색성의 핵을 갖고 있었다. 또한 4 예는 혼합 간세포-담관상피암종의 '이행성' 아형으로 분류하였다. 9 예는 형태학적으로는 거의 전형적인 간세포암종의 소견을 보였으나, 좀더 작은 세포들로 이루어져 있었고 이들은 소세포성 간세포암종으로 분류하였다. 나머지 24 예는 20 예

의 전형적인 간세포암종과 4 예의 전형적인 담관상피암종으로 분류하였다. 면역조직화학적 염색을 시행한 결과, 10 예의 '중간형'(간세포-담관상피세포) 암종 중 6 예에서는 간세포 및 담관상피세포 표지자가 동시에 발현되었으며, 6 예는 *c-kit* 염색에 양성을 나타냈다. 4 예의 '이행성' 혼합 간세포-담관상피암종 중에서는 2 예가 간세포와 담관상피세포의 표지자를 동시에 발현하였고, 이 2 예 중에서 1 예에서 *c-kit* 를 발현하였다. 소세포성 간세포암종 중 3 예에서 간세포와 담관상피세포의 표지자가 모두 양성이었으나 *c-kit* 를 발현하는 예는 없었으며, 나머지 6 예에서는 모두 *c-kit* 를 발현하였으나 담관상피세포 표지자를 발현하는 예는 없었다. 전형적인 간세포암종은 모두 간세포 표지자만 발현하였으며, 전형적인 담관상피암종은 모두 담관상피세포 표지자만 발현하였다.

이상의 결과로 '중간형' (간세포-담관상피세포) 간암종은 간세포와 담관상피세포로의 이중분화능력을 갖는 전구세포로부터 기원한 간암종으로 특별한 아형으로 생각된다.

핵심되는 말: 간세포암종, 담관상피암종, 혼합형 간세포-담관상피암종, *c-kit*, 간모세포, 중간형