

The Impact of Histological Differentiation in Patients with HCC

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the third most common cause of cancer death worldwide[1]. HCC is closely related to cirrhosis, secondary to alcoholism, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. HCC is a heterogeneous disease with respect to clinical behaviour and histological features. There is a variable degree of differentiation, from well-differentiated tumours which closely resemble hepatocytes, to poorly differentiated tumours with more aggressive cytological features and loss of cellular architecture. A subset of HCC display concomitant differentiation towards biliary epithelium, and are known as combined hepatocellular-cholangiocarcinoma (cHCC-CC). In this chapter, we review the significance of the histological variations encompassed by HCC.

Morphology

By gross morphology, HCC are classified into massive, nodular, and diffuse types. The massive type consists of a large dominant tumour. The nodular type is most common, and comprises multiple tumour nodules, either due to multicentric synchronous tumours, or as a result of intrahepatic metastases. Tumours are surrounded by distinct fibrous capsules. Microscopic invasion of capsule is frequently observed. In contrast, in the diffuse type, an infiltrative growth replaces liver parenchyma, and the patient presents with hepatomegaly and liver decompensation. HCC have a strong propensity to venous invasion, giving rise to portal vein thrombosis and rapid progression of portal hypertension.

DEGREE OF DIFFERENTIATION

Hepatic carcinogenesis

The sequence of hepatic carcinogenesis is composed of induction, promotion and progression [2]. Induction is carcinogen induced DNA mutation. Viral and alcoholic hepatitis are common processes involved in induction. Induction is followed by promotion, which is clonal proliferation of induced cells. The increased proliferation is compensated by increased apoptosis, and the overall architecture is maintained. Progression is characterized by accumulation of further DNA damage leading to decreased apoptosis. Without regulated cell death the clonal expansion progress to become an invasive malignancy.

In the early phase, the tumours are uniformly well-differentiated. As tumour growth progresses, certain areas attain further mutations and dedifferentiate [3]. Dedifferentiated malignant hepatocytes replace the surrounding tumour, develop vascular invasion and metastasize. This theory is supported by several concordance clinical observations. Small HCC of 1-2cm in size are uniformly well-differentiated [4]. Larger tumours have a heterogeneous 'tumour within a tumour' appearance, of which less differentiated foci are surrounded by better differentiated malignant hepatocytes [5]. This phenomenon is known as grading heterogeneity. Larger tumours are of higher grades and are more heterogeneous with respect to grading.

Edmondson and steiner schema

HCC are graded with the Edmondson and Steiner schema [6] from I to IV (Table 1). Assessment is based on cytological features (quantity of cytoplasm, size and degree of hyperchromatism of the nuclei), cellular architecture and evidence of cell function i.e. bile production. Grade I (Figure 1 and Figure 2) refers to a distinct area within a tumour, from which tumour cells are virtually indistinguishable from hyperplastic hepatocytes, and the diagnosis of HCC rests upon observing other higher grade areas of the tumour. In Grade II (Figure 3 and Figure 4), tumour cells have large and hyperchromatic nuclei with abundant cytoplasm. Trabecular pattern is observed, where tumour cells are separated by sinusoids. Acini are frequent and bile formation is evident. In Grade III tumour (Figure 5 and Figure 6), nuclei are larger, more hyperchromatic and occupy a

greater proportion of the cell than in Grade II. Acini are less frequent and might not contain bile. In Grade IV (Figure 7 and Figure 8), tumour cells have intensely hyperchromatic nuclei and scanty cytoplasm. Trabeculae and acini are not found.

Table 1: Edmondson and Steiner schema for HCC differentiation.

Grade	Description
I	Refers to areas in grade II carcinomas where the difference between the tumour cells and hyperplastic liver cells is so minor that diagnosis of carcinoma rests upon the demonstration of more aggressive growths in other parts of the neoplasm
II	Nuclei are larger and more hyperchromatic than normal, but the cytoplasm is abundant and acidophilic. Acini were frequent, their lumina varying in size from tiny canaliculi to large thyroidlike spaces. The lumina are often filled with bile
III	Nuclei are usually larger and more hyperchromatic than grade II. These nuclei occupied a relatively greater proportion of the cell. The cytoplasm is granular and acidophilic as in grade II but usually less so. Sometimes there is segregation of the granular cytoplasmic material toward the end of the cell bordering a lumen that usually do not contain bile
IV	Nuclei are intensely hyperchromatic and occupied the greater part of the cell. The cytoplasm is often scanty, and contained fewer granules. Trabeculae are difficult to find, and many of the cell masses seemed to lie loosely without cohesion in vascular channels

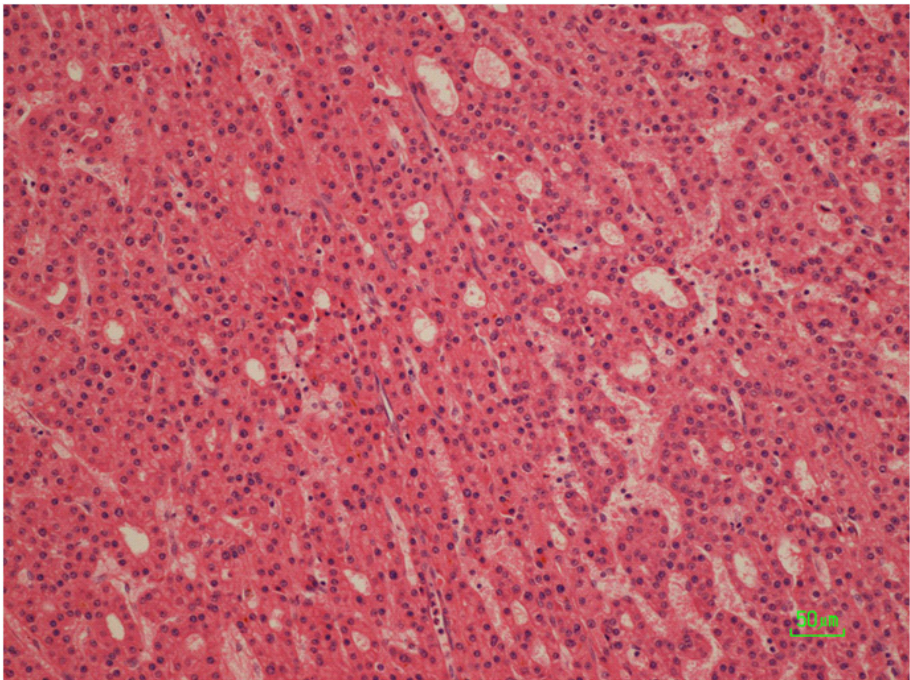


Figure 1: Grade I HCC (100x, H&E staining).

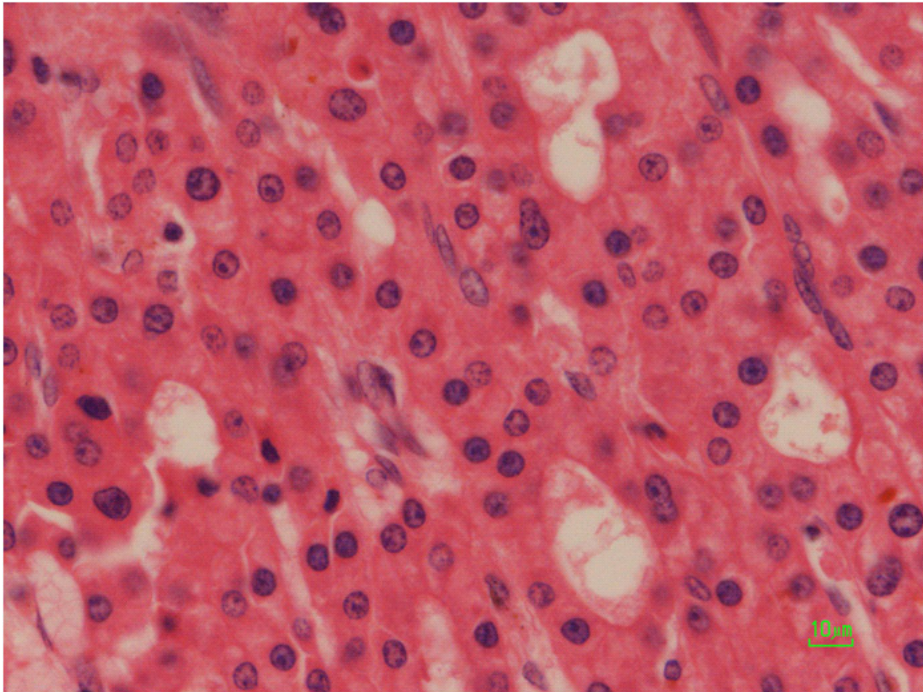


Figure 2: Grade I HCC (400x, H&E staining).

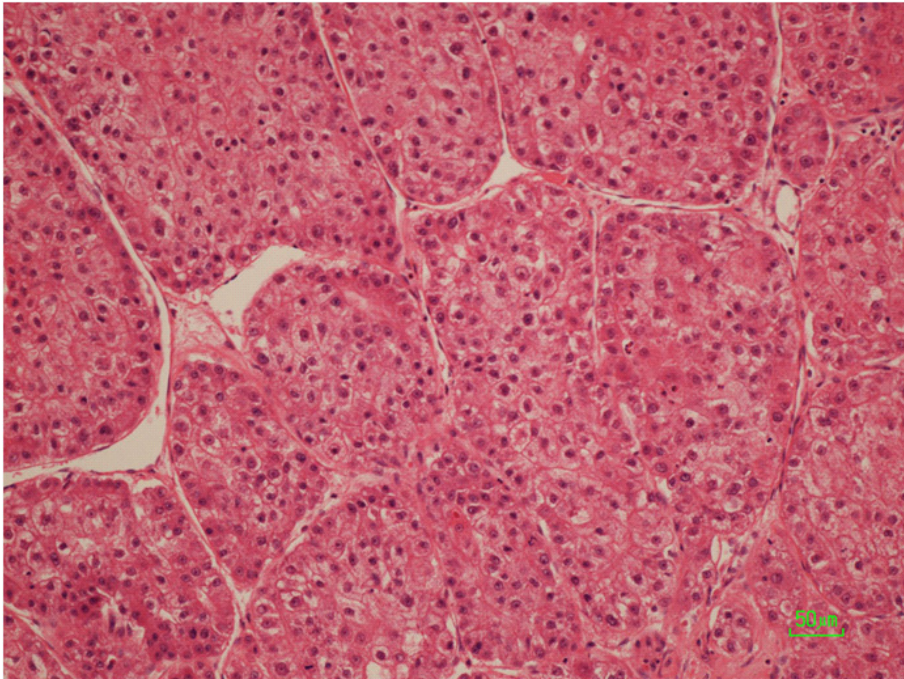


Figure 3: Grade II HCC (100x, H&E staining).

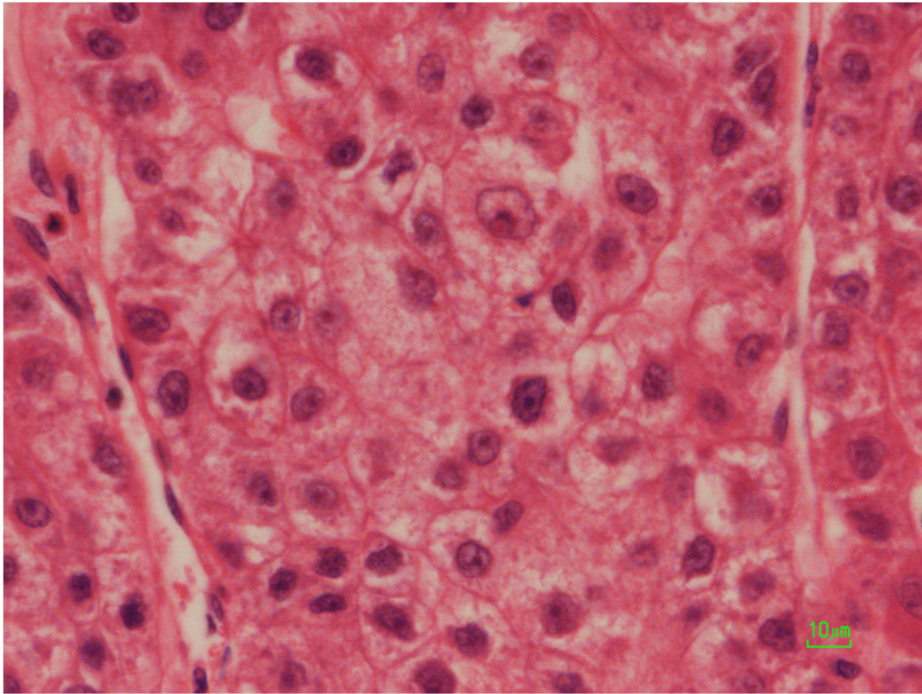


Figure 4: Grade II HCC (400x, H&E staining).

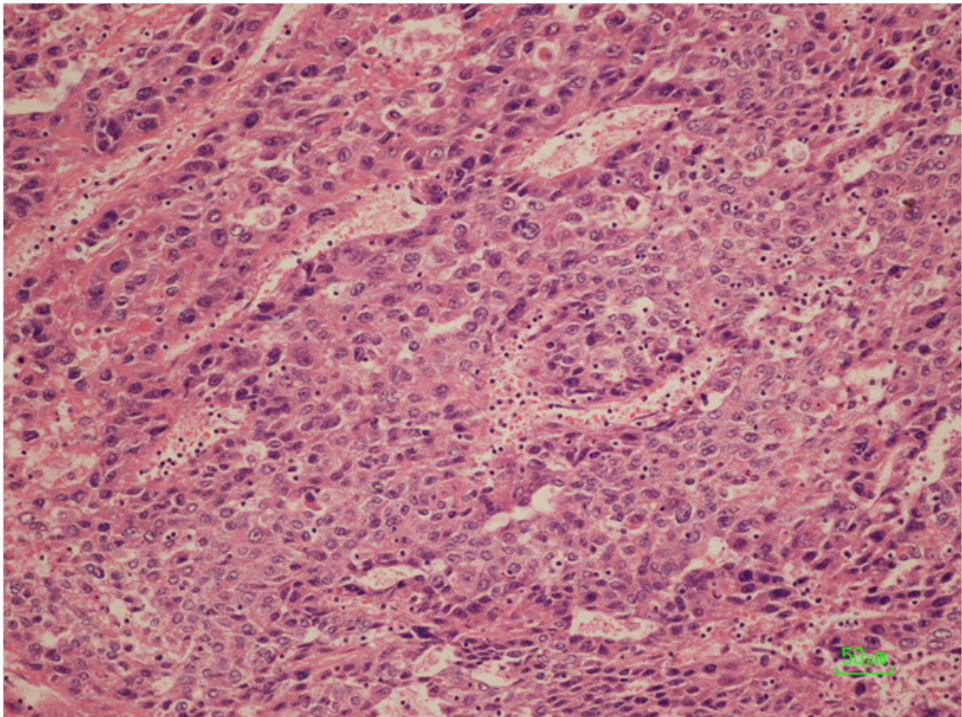


Figure 5: Grade III HCC (100x, H&E staining).

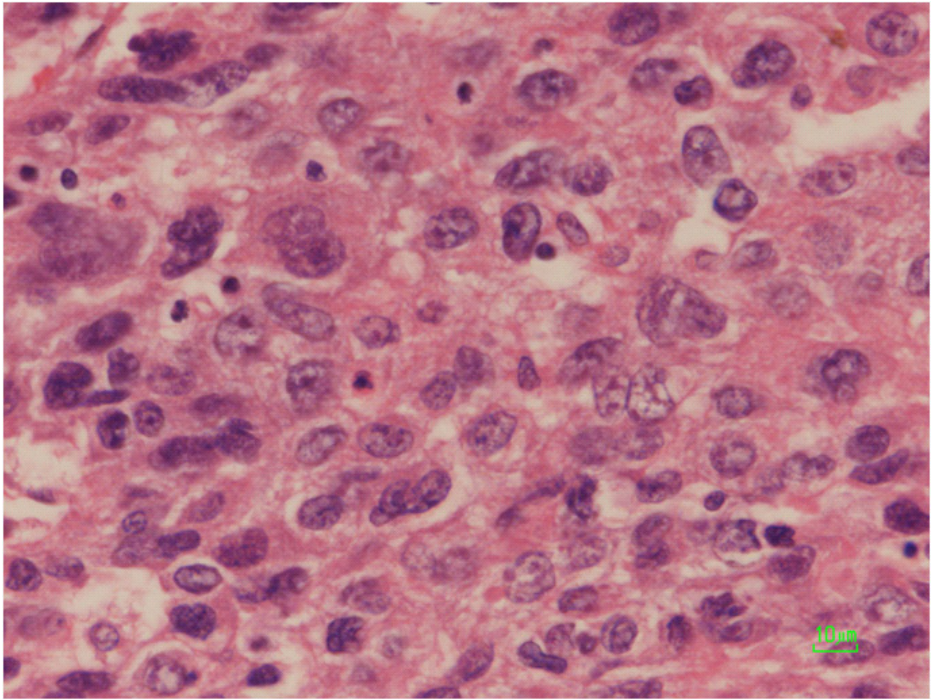


Figure 6: Grade III HCC (400x, H&E staining).

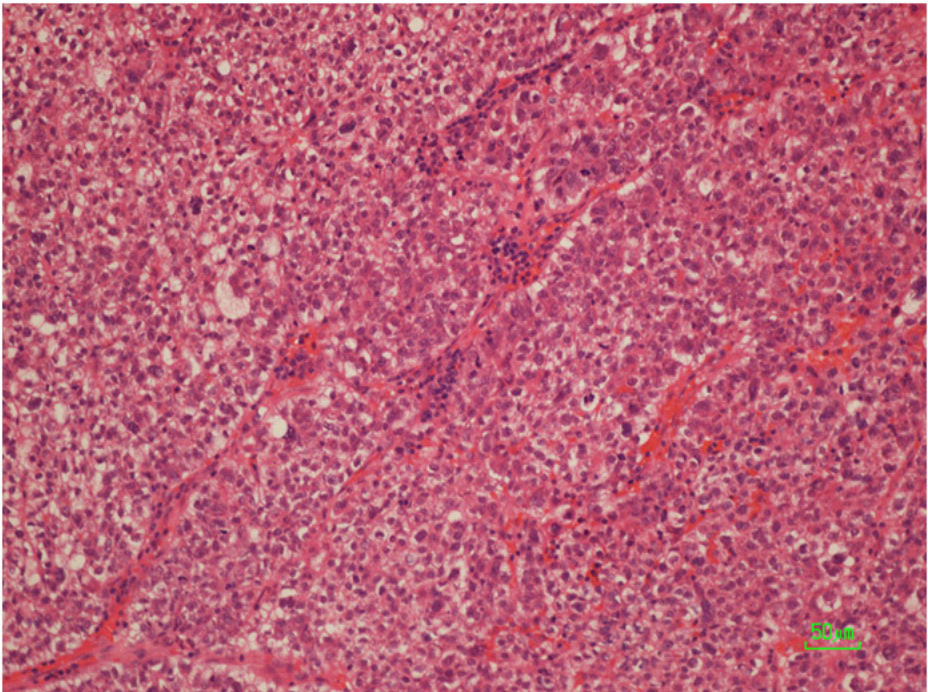


Figure 7: Grade IV HCC (100x, H&E staining).

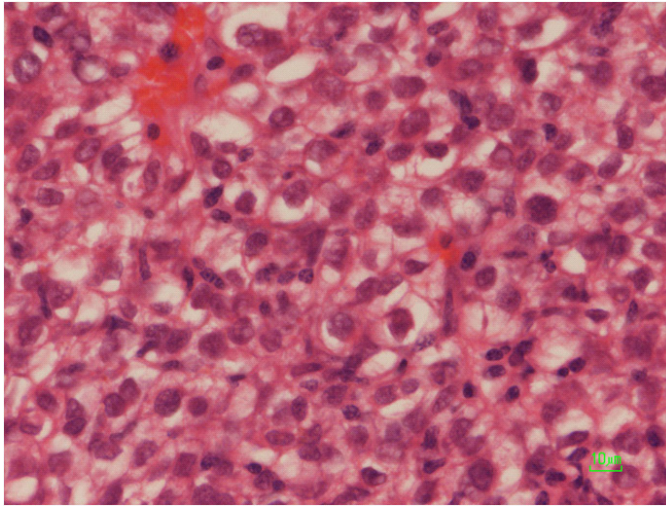


Figure 8: Grade IV HCC (400x, H&E staining).

WHO Grading

In the presence of grading heterogeneity, tumour behaviour is determined by the worst grade [7,8]. Therefore Edmondson Grade I is seldom representative. The World Health Organisation (WHO) recommended a three-tier grading system, classifying HCC into well-, moderately and poorly differentiated [9], broadly equivalent to Edmondson Grade II, III and IV (Table 2), and has gained wide acceptance.

Table 2: WHO grading for HCC differentiation.

Well-differentiated	Cells with mild atypia and increased nucleus-to-cytoplasm ratio arranged in a thin trabecular pattern, with frequent pseudoglandular or acinar structures.
Moderately differentiated	Trabecular growth of three or more cells in thickness. Tumour cells have abundant eosinophilic cytoplasm and round nuclei with distinct nucleoli. A pseudoglandular pattern is frequent, and pseudoglands often contain bile or proteinaceous fluid.
Poorly differentiated	Solid pattern growth without distinct sinusoid-like blood spaces; only slit-like blood vessels are observed in large tumour nests. The neoplastic cells show an increased nucleus, cytoplasm ratio, and frequently pleomorphisms, including bizarre giant cells.

Molecular Aspect of Grading and Cancer Stem Cell Theory

Advances in molecular biology have allowed identifications of molecular markers for HCC differentiation. Hepatocyte paraffin 1 (HepPar 1) (Figure 9 and Figure 10), polyclonal carcinoembryonic antigen and Cluster of Differentiation 10 (CD10) are markers of hepatocellular differentiation (Table 3). Cluster of Differentiation 133 (CD133), Cluster of Differentiation 90 (CD90) and Epithelial Cell Adhesion Molecule (EpCAM) positivity are associated with poorly differentiated HCC [10]. Of note, the markers of high grade tumours are initially identified as molecular markers of hepatic stem cells. They are characterized by the capacities of self-renewal and multi-lineage differentiation, into hepatocyte and biliary epithelial cell, and are responsible for the unique regenerative capacity of the liver.

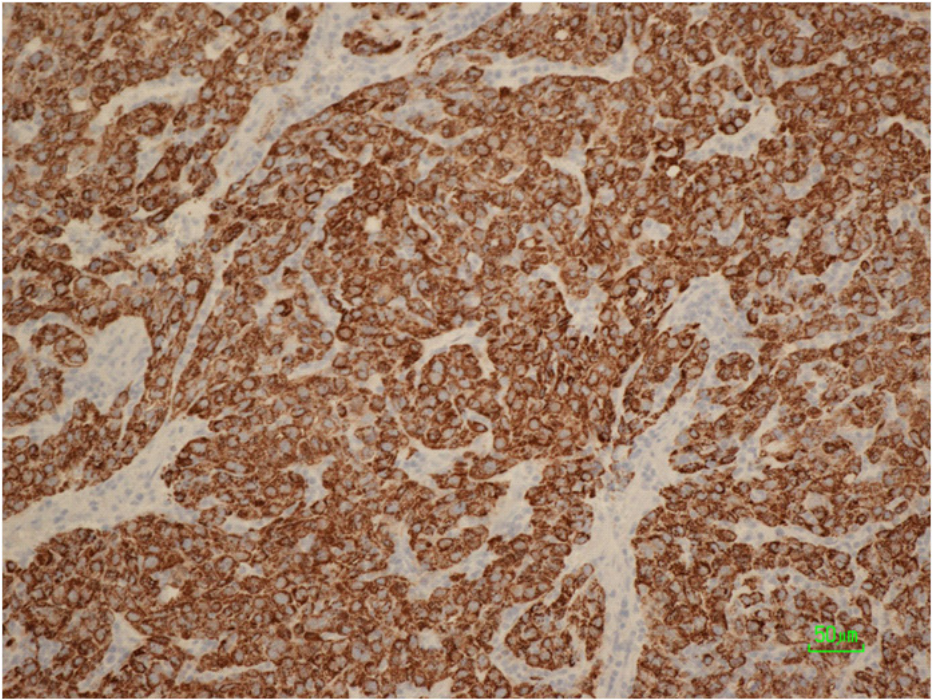


Figure 9: Grade IV HCC (100x, HepPar1 staining).

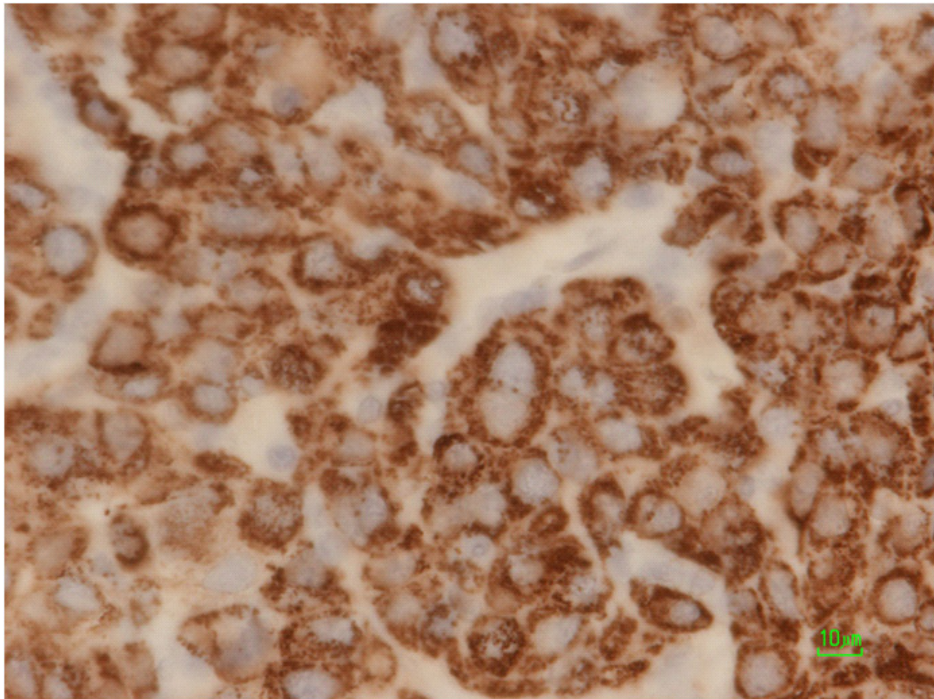


Figure 10: Grade IV HCC (400x, HepPar1 staining).

Table 3: Common molecular markers of HCC differentiation.

Hepatocellular differentiation	HepPar1, polyclonal carcinoembryonic antigen, CD10
Biliary differentiation	CK7, CK19
Hepatic stem cell markers	CD133, CD90, EpCAM

Cancer stem cells results when mutation and malignant transformation occurs in hepatic stem cells. Cancer stem cells remain undifferentiated, and retain its pluripotency. This explains the association between cancer stem cells and poorly differentiated HCC, and also combined hepatocellular-cholangiocarcinoma (cHCC-CC), which is discussed in the later paragraphs. Studies showed that a subpopulation of cancer stem cells can be detected in 28-50% of HCC [11,13], and presence of these cancer stem are associated with poor grade and inferior survival [14]. Considering the prognostic implications, the traditional histological classification of these tumours as HCC with cancer stem cells has been challenged. From biological point of view, these tumours are better considered as malignancy of hepatic stem cells, differentiating towards the spectrum of HCC. A transition of histology to molecular biology in pathological diagnosis has prognostic implications and guides clinical management. A similar but more established transition is seen in breast cancer, of which adjuvant therapy is now guided by tumour biology, including tumour grade, hormonal receptor status and proliferation indexes.

Grading and Prognosis

Hepatic resection

Hepatic resection is the primary curative treatment for HCC, and therefore the most important prognostic feature is resectability. It is predominantly determined by tumour stage (multifocality, portal venous invasion, distant metastasis), liver function and patient comorbidity. The widely used Barcelona staging system incorporates these essential determinants of prognosis [15]. Resectable HCC has a 5-year survival of 50%, as compared to 10% of unresectable disease.

However, grading predicts prognosis after resection. Long term survivals are observed in patients with large or multiple, but well-differentiated tumours [16]. In contrast, high grade tumours, sometimes despite smaller, are prone to recurrence. Poor differentiation is independently associated with microvascular invasion [17,18], which is a prerequisite for systemic dissemination and an important predictor of postoperative survival [19]. Wayne reported a median survival of 4.9 years for resectable grade I and II disease, and 3.5 years for grade III and IV [20].

In heterogeneously graded tumours, the worst grade, rather than the major grade, determines the prognosis [7,8]. Inferior prognosis has prompted more aggressive therapy. However, efficacies of adjuvant therapy, including trans-arterial chemoembolization (TACE) and systemic therapy such as chemotherapy and targeted therapy, have been disappointing. Nevertheless, a higher chance of recurrence should prompt the clinician to more aggressive surveillance to obtain a higher chance of re-intervention.

Liver transplantation

Very few patients present with resectable diseases. Firstly, development of HCC follows cirrhosis in 80% of cases [21], thereby an inadequate hepatic reserve forbids resection. Secondly, 75% of the patients have multifocal disease upon presentation [22]. Even after a successful resection, recurrence is frequent due to persistence of cirrhosis. Liver transplantation provides an attractive alternative to resection as it treats HCC and cirrhosis simultaneously.

Patient selection is of utmost importance. Initially, liver transplantation was offered to patients with locally advanced and therefore unresectable tumours. The disappointing outcomes have led transplant surgeons to select transplant candidate based on predicted prognosis [23]. Post-transplant recurrences progress rapidly under immunosuppression, and are virtually incurable. Good predicted survival is mandatory to justify the allocation of a scarce cadaveric liver graft or the morbidity imposed on the living donor.

Number and size of tumour predict outcome and form the basis of current selection criteria. The Milan's criteria restricts transplant candidate to those with single tumour less than or equal to 5cm, or up to three tumours less than or equal to 3cm in diameter [24]. The University of California San Francisco (UCSF) criteria is single tumour less than or equal to 6.5cm or up to three tumours with the largest diameter less than or equal to 4.5cm and total diameter not exceeding 8cm [25]. The implementation of these stringent morphological criteria has brought the 5-year survival to 80% [25].

Tumour histology also carries significant prognostic implications. Studies have shown that under current selection criteria, microvascular invasion is one of the strongest predictors of tumour recurrence after liver transplantation [16,24,26,27]. Poor differentiation is an independent predictor of microvascular invasion [17,18]. Poorly differentiated HCC is associated with a 40% 5-year post-transplant survival, compared to 70-80% in moderately and well-differentiated tumours [17]. Several groups have proposed to incorporate tumour biology into selection criteria [28,29], and to exclude high grade tumours from liver transplantation. An accurate preoperative evaluation of tumour grade is prerequisite.

Prediction of Tumour Grade

Preoperative biopsy

The role of preoperative core biopsy was widely investigated but results have been disappointing. Due to the intrinsic grading heterogeneity of HCC, preoperative biopsy correlates poorly with resection pathology. In a series pre-operative biopsy accurately predicted the tumour grade only in 45% of the cases [18]. Moreover, it is not uncommon for transplant candidates to have more than one tumour. In those cases, preoperative biopsy of the largest tumour may not be representative of the overall tumour burden. Sometimes the largest tumour are well-differentiated, but the smaller tumours are poorly differentiated [30]. Sampling of multiple lesions

may improve accuracy, but the increased procedural risk likely outweighs any additional benefits. Biopsy guided by functional imaging e.g. positron emission tomography-computed tomography (PET-CT) may be a potential direction and the efficacy requires evaluation.

Extensive research work has explored multiple biological markers reflecting tumour behaviour. Markers of hepatic stem cells reflects high grade tumour and suggests vascular invasion [10]. Positivity for molecular pathways of angiogenesis e.g. angiopoietin-2 and hypoxia-induced factor-1 alpha are associated with more recurrences after resection [31]. Proliferating cell nuclear antigen (PCNA) enhances tumour proliferation and predicts poor prognosis [32]. However, pre-existing works are based on resected pathological specimen. Whether these molecular markers can be detected sensitively on a core biopsy remains questionable, and their role in preoperative biopsy requires further studies.

Imaging

In light of the suboptimal performance with preoperative biopsy, many efforts have focused on radiological evaluation of HCC differentiation. HCC has a predominant blood supply from the hepatic artery, while normal liver parenchyma derives their major supply from the portal vein. HCC typically appears as an arterial enhancing lesion with portal venous washout (hypoenhancement when compared with liver parenchyma) on contrast computed tomography (CT) and magnetic resonance imaging (MRI). Along with dedifferentiation, arterial blood supply decreases significantly [33]. Poorly differentiated components appear as non-enhancing area in the arterial phase. On a CT scan, arterial enhancement with non-enhancing areas predicts poorly differentiated HCC with a sensitivity of 85% and a specificity of 76% [34].

The use of PET-CT has evolved the preoperative staging for malignancy in the past decade. It is a functional imaging, which study tissue metabolic activities by measuring uptake of radioisotopes. It detects occult distant metastases for various solid and haematological malignancies. 18-fluorodeoxyglucose (18F-FDG) is a commonly used isotope but is less sensitive for HCC. In our locality, it is coupled with carbon-11 acetate (11C-acetate) when imaging HCC [35]. Interestingly, 18F-FDG uptake correlates with tumour grade [36]. Poorly differentiated HCC have higher 18F-FDG but similar 11C-acetate uptake compared with more differentiated counterparts. In addition, higher 18F-FDG uptake translates to worse outcome with shorter survival. Magnetic resonant imaging (MRI) with diffusion weighted imaging (DWI) is another recent advance in HCC imaging, which evaluate of tumour grade through assessing tumour cellularity. DWI assesses the random motion of water molecules in tissue. Increased cellularity and decreased extracellular spaces in a high grade tumour restrict water diffusion, and appear as hyperintensity on DWI. In a meta-analysis, signal intensity on DWI is found to be sensitive (sensitivity 84%) but not specific (specificity 48%) for poorly differentiated tumour [37].

Alpha-fetoprotein

Alpha-fetoprotein (AFP) is a human foetal plasma protein and a tumour marker for HCC. A high level of serum AFP correlates not only with larger tumour load, but also with higher tumour grade and microvascular invasion [38]. Serum AFP level provides an attractive alternative for estimation of tumour grade because of its simplicity. In addition, its prognostic value has been verified. Researchers have suggested using AFP value greater than 400 ng/mL as a criterion to predict poor differentiation [39]. Transplant surgeons have developed patient selection criterion based on serum AFP concentration [27]. The Hangzhou criteria was established as total tumour diameter less than or equal to 8 cm or total tumour diameter more than 8 cm, graded I or II and AFP level less than or equal to 400 ng/mL. Based on the Hangzhou criteria, survivals have been reported to be comparable with other well established criteria.

Specific radiological features and serum levels of AFP correlate with histological grade of HCC. They might provide attractive alternative to preoperative biopsy, which is not only invasive but also unreliable. However, further studies are warranted before specific radiological and biochemical parameters can be incorporated into selection criteria for liver transplantation.

Variants of HCC

Apart from assessing the tumour grade, histological evaluation allows identifications of specific variants of HCC, based on their specific cytological features. They include clear cell, fibrolamellar, sacromatoid, sclerosing and chCC-CC [9]. Clear cells are large cells with clear cytoplasm, due to abundant cytoplasmic glycogen or lipid that is not stained by haematoxylin or eosin. Clear cell HCC are of low grade and carry better prognosis [40,41]. Sarcomatoid HCC have mesenchymal differentiation evident by presence of spindle-shaped tumour cells. It mimics liver abscess in its clinical course, with fever and leucocytosis, therefore is also known as febrile type HCC. Sclerosing HCC is characterized histologically by intense fibrosis. This rare variant is associated with hypercalcaemia secondary to tumour production of parathyroid hormone-related protein [42]. Fibrolamellar type of HCC and chCC-CC are distinctive variants of HCC with respect to their histology and prognosis and they are discussed in the following paragraphs.

Fibrolamellar carcinoma

After observing a 14-year-old long term survivor of HCC, Edmondson described fibrolamellar HCC as a pathological variant [42]. Perhaps of more importance is its distinct clinical behaviour. Fibrolamellar HCC primarily occurs in young Caucasians without underlying liver cirrhosis. The median onset age is 33, compared to 66 of classical HCC [43]. It is rarely seen in the East, where classical HCC is endemic due to HBV infection. It appears as a solitary and well-circumscribed tumour in the background of a non-cirrhotic liver. Due to normal liver function, resection is usually feasible and there is a comparably better prognosis, which explains why it is considered as a distinct clinical entity. Tumour growth is usually rapid and recurrence is common, and it would be inaccurate to consider fibrolamellar HCC to be an indolent tumour.

Histologically, fibrolamellar HCC is characterized by sheets of large polygonal cells interspersed by laminated layers of fibrous stroma, which connects to form a central scar. A non-enhancing central scar can be appreciated in the venous phase of contrast CT scan. Hallmark cytological features include abundant eosinophilic cytoplasm, large vesiculated nuclei, and large nucleoli [44]. In terms of tumour grade, fibrolamellar HCC is moderately differentiated. Fibrolamellar HCC is associated with focal nodular hyperplasia (FNH), and they share clinical and histological features like central scarring. This has prompted researchers to investigate their causal relationship [45], but so far no definite conclusions have been reached. Findings of nodular hyperplasia surrounding fibrolamellar HCC suggest FNH may be a secondary reaction to the primary tumour.

The molecular profile of fibrolamellar HCC is also unique. The presence of normal liver suggests that development of fibrolamellar HCC does not result from accumulation of multiple DNA damage due to chronic liver disease. This is evident by absence of classical molecular markers for HCC, including AFP and TP53 mutations. Fibrolamellar HCC stains negative for AFP, and does not secrete AFP into systemic circulation. Elevation of AFP should raise the concern of alternative diagnosis. Immunohistochemical staining helps to confirm the diagnosis. Fibrolamellar HCC uniformly stains positive for Cluster of Differentiation 68 (CD68) [46] and cytokeratin-7 (CK7), a marker of biliary differentiation.

Combined hepatocellular-cholangiocarcinoma (cHCC-CC)

Primary liver cancers are classified into HCC, which develops from hepatocytes, and cholangiocarcinoma (CC), which derives from bile duct epithelium. Combined hepatocellular-cholangiocarcinoma (cHCC-CC) was first described by Allen and Lisa in 1949 [47] as a tumour with mixed histological elements of HCC and CC. It is rare and comprises 1-5% of primary hepatic malignancy. The aetiology is unknown. Clinical association with chronic liver disease has been observed, but largely depends on the local prevalence of chronic HBV infection. Asian centres reported 40-50% of their cHCC-CC series with background viral cirrhosis [48,49], while in a Western series, only 13% of coexisting cirrhosis [50].

PATHOLOGY

Classical HCC displays carcinoma cells arranged in trabecular architecture with bile in canaliculi. CC typically forms glandular structures and produces mucin. cHCC-CC is diagnosed by a combined histological pattern of hepatocellular and biliary epithelial components (Figures 11-14). A predominant differentiation, to either HCC or CC, may be identified. cHCC-CCs developing from cirrhosis have a higher resemblance to HCC.

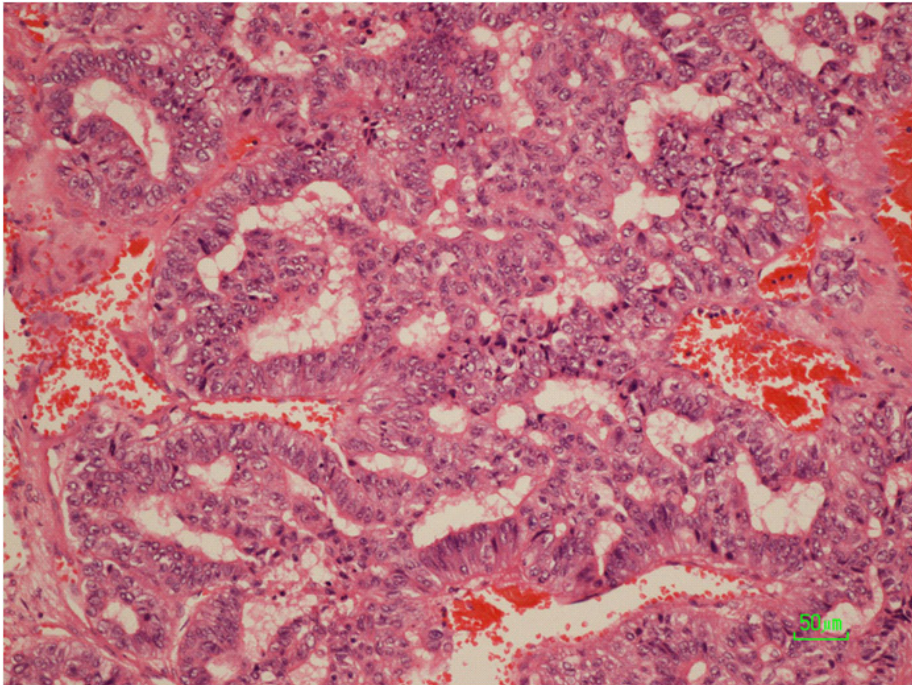


Figure 11: chHCC-CC. CC area (100x, H&E staining).

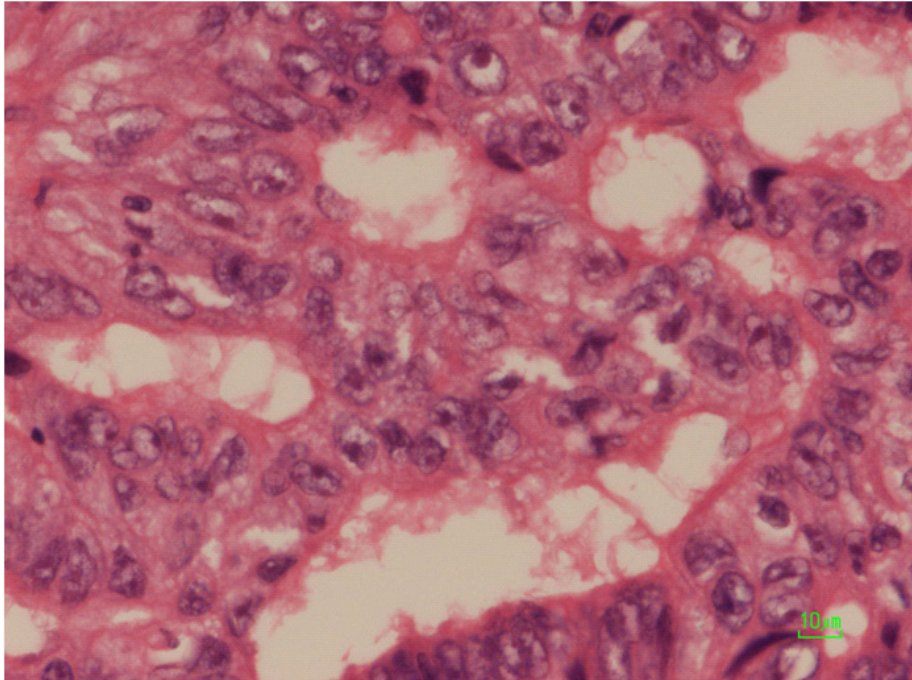


Figure 12: chHCC-CC. CC area (400x, H&E staining).

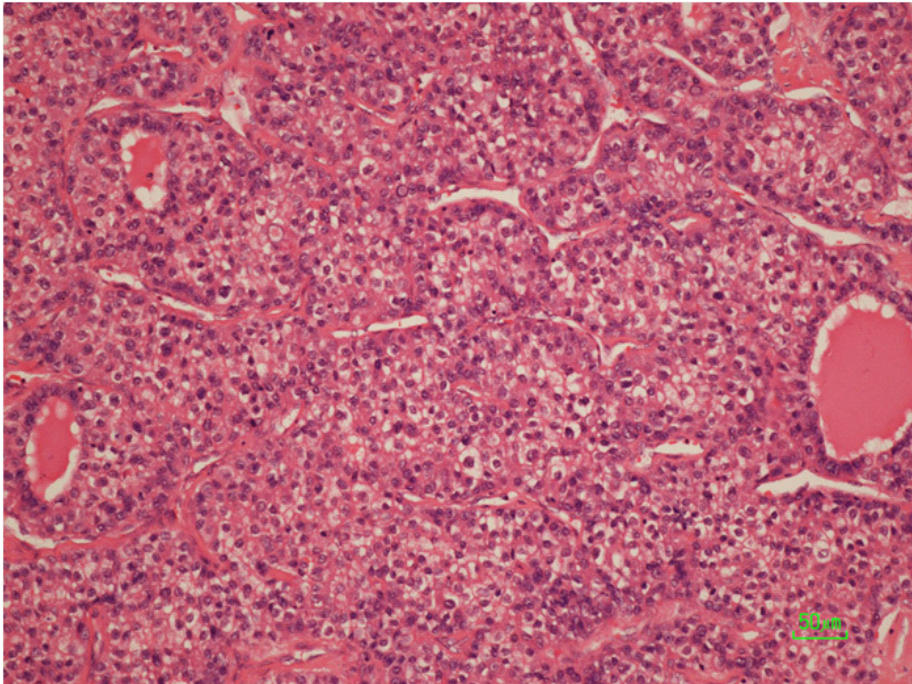


Figure 13: cHCC-CC. HCC area (100x, H&E staining).

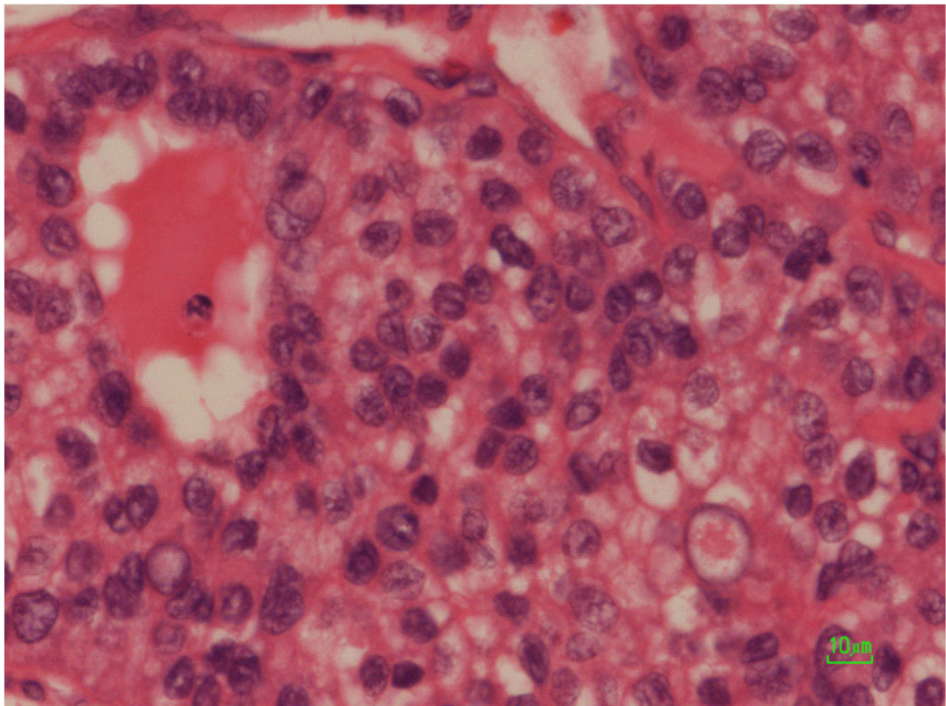


Figure 14: cHCC-CC. HCC area (400x, H&E staining).

Identification of bile and detection of mucin by a mucin stain confirms the diagnosis. In equivocal cases, immunohistochemical staining helps to recognize the presence of dual differentiation. Positive staining for HepPar 1 (Figure 15 and Figure 16), polyclonal carcinoembryonic antigen and CD10 indicates hepatocellular differentiation, while CK7 and cytokeratin 19 (CK19) stain for biliary differentiation (Figures 17-20) [51]. However, a diagnosis solely depending on immunohistochemistry cannot be justified, as markers of biliary differentiation can be found much more frequently than histological findings of cHCC-CC are evident [52]. Their importance will be further discussed in next session. After all, in equivocal cases, electron microscopy helps to confirm the diagnosis by appreciating cellular features of dual lineage. Abundant mitochondria and rough endoplasmic reticulum suggests hepatocellular differentiation. Microvillus projection into luminal surface indicates biliary differentiation.

Several hypotheses exist for pathogenesis of cHCC-CC. 1) Collision tumour, of which cHCC-CC develops from incidental coexistence of both tumours. The findings of interspersed elements of both lineages suggest against this theory. 2) Subsequent de-differentiation to the other tumour. 3) Cancer stem cell theory. The pluripotent hepatic stem cells undergo malignant change, while maintaining the ability of both hepatocellular and biliary differentiation. Experiments showing cell lines from cHCC-CC can differentiate into HCC or CC under different conditions supported this theory [53].

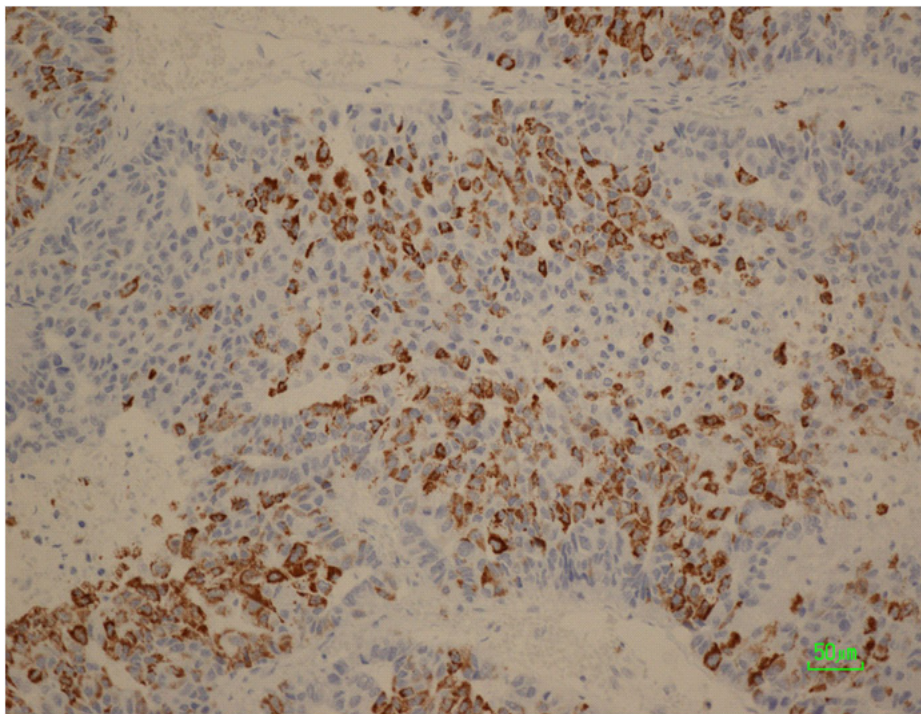


Figure 15: cHCC-CC. (100x, HepPar1 staining).

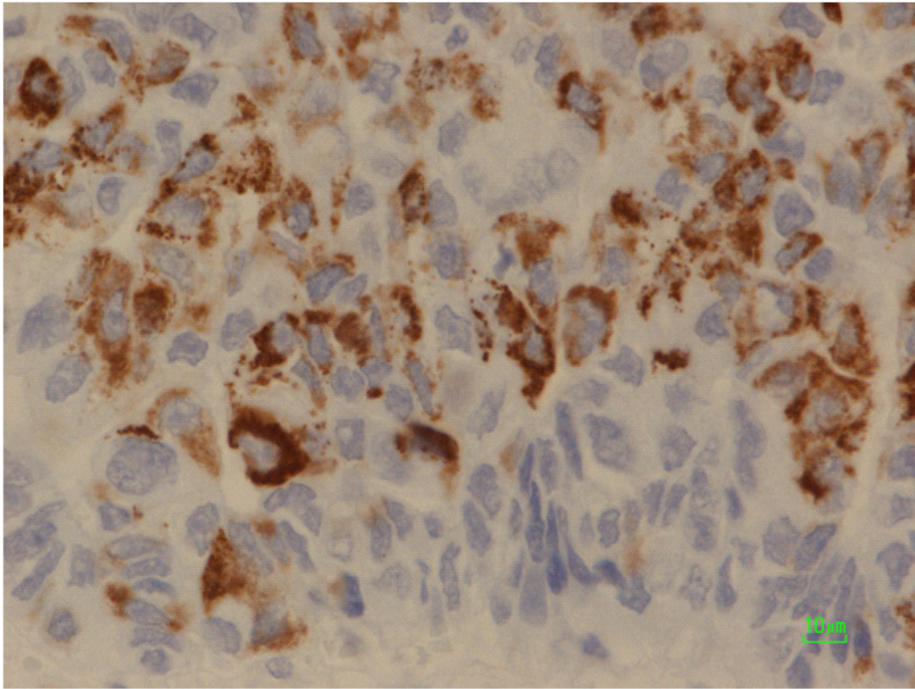


Figure 16: cHCC-CC. (400x, HepPar1 staining).

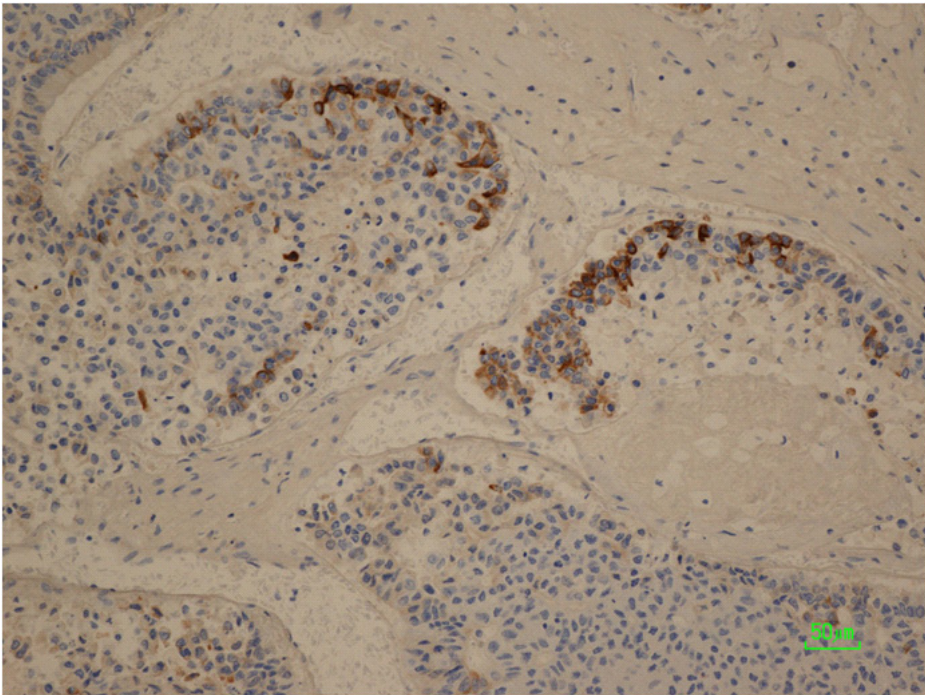


Figure 17: cHCC-CC. (100x, CK7 staining).

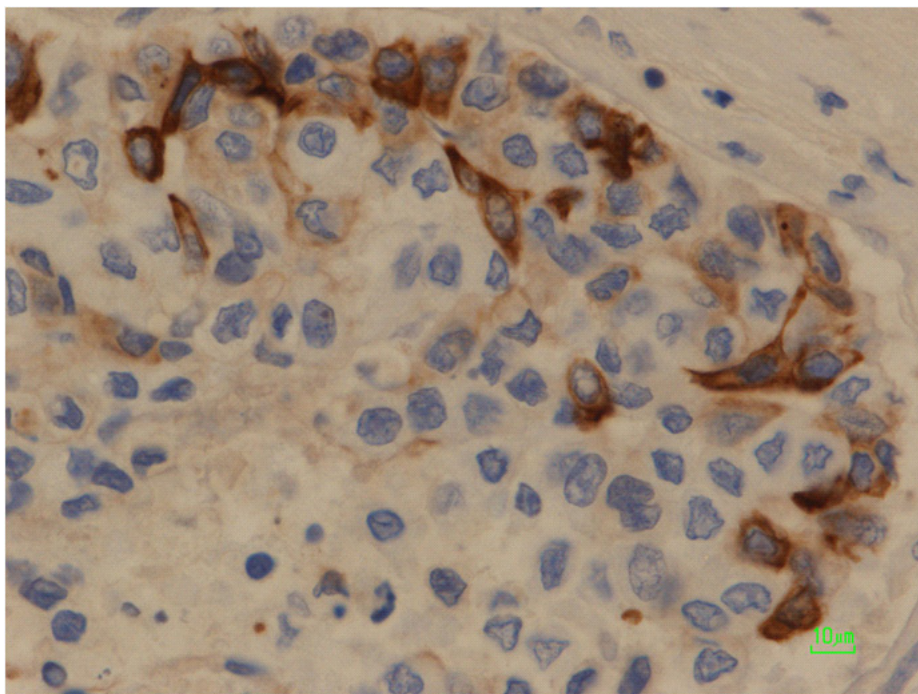


Figure 18: chCC-CC. (400x, CK7 staining).

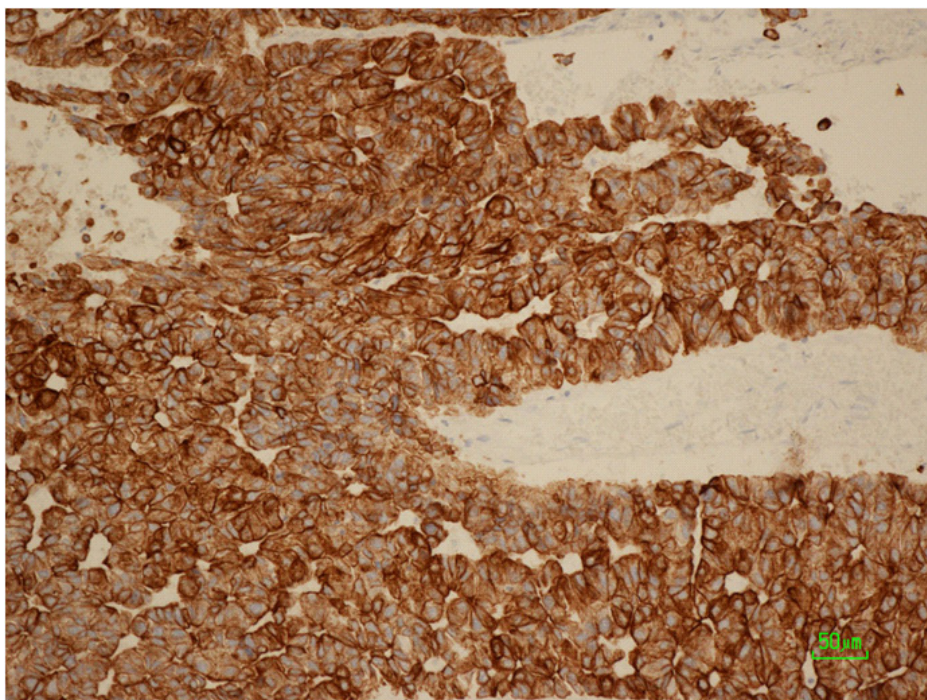


Figure 19: chCC-CC. (100x, CK19 staining).

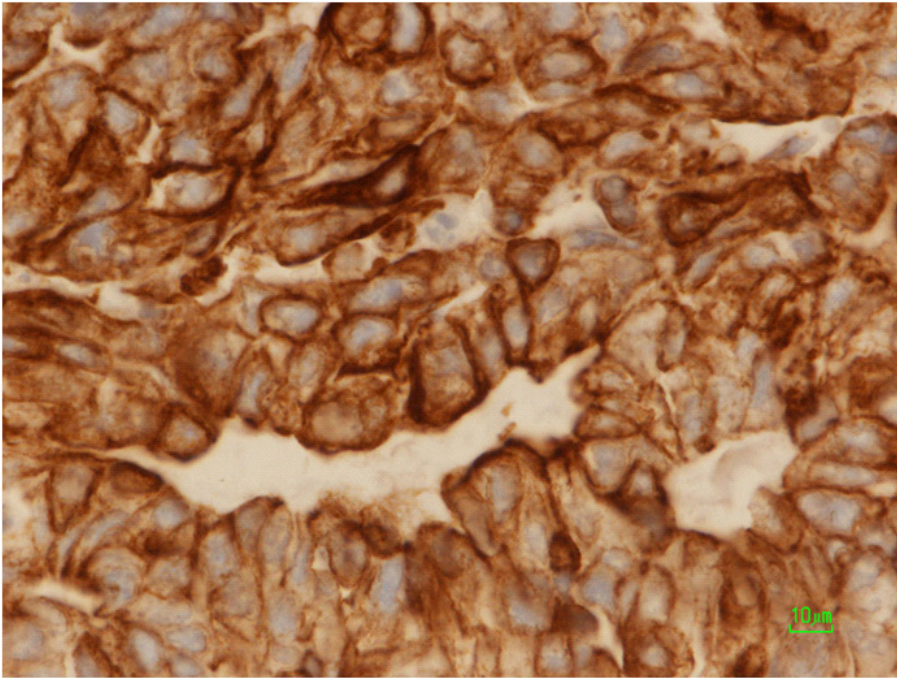


Figure 20: chHCC-CC. (400x, CK19 staining).

CLINICAL IMPLICATIONS

The survival of chHCC-CC after resection is poorer when compared to HCC and CC [50,54]. In our centre, the median disease-free survival for resectable chHCC-CC is 10 months, compared to 18 months and 24 months of HCC and CC [55]. Liver transplantation may have a role in selected patients [56], but outcome data is few. chHCC-CCs have more vascular invasion than typical HCC [57]. More importantly, chHCC-CC is associated with lymph node metastasis, as with CC, mandating a portal lymph node dissection and highlighting the importance of establishing a preoperative diagnosis [57]. Pre-operative pathological confirmation is difficult due to sampling error, as in the case of pre-operative HCC grading. Given the rarity of this tumour, the pre-operative diagnosis depends on high index of suspicion. An elevated serum level of carcinoembryonic antigen (CEA) and atypical radiological features suggest the presence of biliary differentiation. HCC have arterial enhancement and portal venous washout, while CCs are enhanced in the portal venous phase. For chHCC-CC, there is typically arterial enhancement due to the HCC components. In the portal venous phase, the appearance is heterogeneous due to enhancement of CC but washout of HCC components [58]. A predominant differentiation may influence the radiological findings.

With the recent advance in immunohistochemistry, chHCC-CC has been increasingly recognized. In the series by Durnez, 28% of their transplanted HCC showed molecular features of biliary differentiation i.e. CK9 and CK17 positivity [52]. Whether this represents an underdiagnosed

population of cHCC-CC with predominant HCC differentiation is debatable. Up to nowadays it is still widely accepted that cHCC-CC is diagnosed on histopathology by demonstration of bile and mucin production. However, the clinical implications of this population cannot be unaddressed. Indeed HCC with molecular features of biliary differentiation have a worse prognosis [52]. Perhaps cHCC-CC and HCC with molecular features of biliary differentiations can both be regarded as tumour arising from hepatic stem cell, but with different spectrum of histological differentiation. In fact, positivity of hepatocellular or hepatic stem cell markers is not uncommonly seen in CC as well. A shift of paradigm from histological diagnosis to molecular classification may have therapeutic and prognostic implications and will be future areas of investigation.

CONCLUSION

Pathological evaluation of HCC allows assessment of tumour grade and identification of specific pathological variants. Tumour grade predict prognosis after resection and liver transplantation. High grade tumours are associated with microvascular invasion, which significantly affects survival. It is difficult to accurately assess tumour grade before resection or transplantation. However preoperative evaluation of tumour grade provides valuable information for selection of transplant candidates. Efforts have been made to incorporate tumour grade into selection criteria because of its prognostic implication. The results of preoperative biopsy has been disappointing, owing to intrinsic grading heterogeneity within a tumour. CT, PET-CT and MRI with DWI all provide circumferential evidence for poorly differentiated tumours.

Specific pathological variants of HCC have distinct clinical behaviour. Fibrolamellar HCC develops in normal livers of young adult, and is characterized by a better prognosis due to better resectability. cHCC-CC display dual differentiation towards hepatocyte and biliary epithelium. Lymphatic metastases are more frequent and overall prognosis is worse. Variants of HCC have specific molecular markers. Recent researches in molecular biology supported the cancer stem cell theory. The presence of hepatic stem cell markers in high grade HCC and biliary markers in cHCC-CC indicates that a pluripotent cancer stem cell with pluripotent differentiation potential could be the precursor lesion in both cases.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer*. 2001; 94: 153–156.
2. Farber E. Hepatocyte proliferation in stepwise development of experimental liver cell cancer. *Dig Dis Sci*. 1991; 36: 973–978.
3. Kojiro M, Nakashima O. Histopathologic evaluation of hepatocellular carcinoma with special reference to small early stage tumors. *Semin Liver Dis*. 1999; 19: 287–296.
4. Kenmochi K, Sugihara S, Kojiro M. Relationship of histologic grade of hepatocellular carcinoma (HCC) to tumor size, and demonstration of tumor cells of multiple different grades in single small HCC. *Liver*. 1987; 7: 18–26.
5. Yamamoto K, Takenaka K, Kajiyama K, Shimada M, Shirabe K, Taketomi A, et al. Cell proliferation and cell loss in nodule-in-nodule hepatocellular carcinoma. *Hepatogastroenterology*. 1999; 46: 813–819.

6. Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer*. 1954; 7: 462–503.
7. Han DH, Choi GH, Kim KS, Choi JS, Park YN, Kim SU, et al. The prognostic significance of the worst grade in hepatocellular carcinoma with heterogeneous histologic grades of differentiation. *J Gastroenterol Hepatol*. 2013; 28: 1384–1390.
8. Sasaki K, Matsuda M, Ohkura Y, Kawamura Y, Inoue M, Hashimoto M, et al. In hepatocellular carcinomas, any proportion of poorly differentiated components is associated with poor prognosis after hepatectomy. *World J Surg*. 2014; 38: 1147–1153.
9. Hamilton SR, Aaltonen LA, Kleihues P, Cavennee WK. World health organization. Classification of tumours. Pathology and genetics of tumours of the digestive system. 2000.
10. Liu R, Shen Y, Nan K, Mi B, Wu T, Guo J, et al. Association Between Expression of Cancer Stem Cell Markers and Poor Differentiation of Hepatocellular Carcinoma: A Meta-Analysis (PRISMA). *Medicine (Baltimore)*. 2015; 94: e1306.
11. Mishra L, Banker T, Murray J, Byers S, Thenappan A, He AR, et al. Liver stem cells and hepatocellular carcinoma. *Hepatology*. 2009; 49: 318–329.
12. Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, et al. Identification and Characterization of Tumorigenic Liver Cancer Stem/Progenitor Cells. *Gastroenterology*. 2007; 132: 2542–2556.
13. Suetsugu A, Nagaki M, Aoki H, Motohashi T, Kunisada T, Moriwaki H. Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells, *Biochem Biophys Res Commun*. 2006; 351: 820–824.
14. Ma YC, Yang JY, Yan LN. Relevant markers of cancer stem cells indicate a poor prognosis in hepatocellular carcinoma patients: a meta-analysis. *Eur J Gastroenterol Hepatol*. 2013; 25: 1007–1016.
15. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis*. 1999; 19: 329–338.
16. Vauthey JN, Lauwers GY, Esnaola NF, Do KA, Belghiti J, Mirza N, et al. Simplified staging for hepatocellular carcinoma. *J Clin Oncol*. 2002; 20: 1527–1536.
17. Jonas S, Bechstein WO, Steinmüller T, Herrmann M, Radke C, Berg T, et al. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology*. 2001; 33: 1080–1086.
18. Pawlik TM, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy: implications for transplant eligibility. *Ann Surg*. 2007; 245: 435–442.
19. Regimbeau JM, Abdalla EK, Vauthey JN, Lauwers GY, Durand F, Nagorney DM, et al. Risk Factors for Early Death due to Recurrence after Liver Resection for Hepatocellular Carcinoma: Results of a Multicenter Study. *J Surg Oncol*. 2004; 85: 36–41.
20. Wayne JD, Lauwers GY, Ikai I, Doherty DA, Belghiti J, Yamaoka Y, et al. Preoperative predictors of survival after resection of small hepatocellular carcinomas. *Ann Surg*. 2002; 235: 722–730.
21. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet*. 2012; 379: 1245–1255.
22. Jelic S, Sotiropoulos GC. Hepatocellular carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *J Eur Soc Med Oncol*. ESMO. 2010; 21: v59–v64.
23. Bismuth H, Chiche L, Adam R, Castaing D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. *Ann Surg*. 1993; 218: 145–51.
24. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med*. 1996; 334: 693–699.
25. Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology*. 2001; 33: 1394–403.
26. Tsai TJ, Chau GY, Lui WY, Tsay SH, King KL, Loong CC. Clinical significance of microscopic tumor venous invasion in patients with resectable hepatocellular carcinoma. *Surgery*. 2000; 127: 603–608.
27. Zheng SS, Xu X, Wu J, Chen J, Wang WL, Zhang M. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation*. 2008; 85: 1726–1732.
28. Cillo U, Vitale A, Bassanello M, Boccagni P, Brolese A, Zanusi A, et al. Liver transplantation for the treatment of moderately or well-differentiated hepatocellular carcinoma. *Ann Surg*. 2004; 239: 150–159.
29. De Carlis L, Giacomoni A, Lauterio A, Slim A, Sammartino C, Pirota V, et al. Liver transplantation for hepatocellular cancer: should the current indication criteria be changed? *Transpl Int*. 2003; 16: 115–122.

30. Young RS, Aldiwani M, Hakeem AR, Nair A, Guthrie A, Wyatt J, et al. Pre-liver transplant biopsy in hepatocellular carcinoma: A potential criterion for exclusion from transplantation? *HPB (Oxford)*. 2013; 15: 418–427.
31. Wada H, Nagano H, Yamamoto H, Yang Y, Kondo M, Ota H, et al. Expression pattern of angiogenic factors and prognosis after hepatic resection in hepatocellular carcinoma: importance of angiopoietin-2 and hypoxia-induced factor-1 alpha. *Liver Int*. 2006; 26: 414–423.
32. Suehiro T, Matsumata T, Itasaka H, Yamamoto K, Kawahara N, Sugimachi K. Clinicopathologic features and prognosis of resected hepatocellular carcinomas of varied sizes with special reference to proliferating cell nuclear antigen. *Cancer*. 1995; 76: 399–405.
33. Asayama Y, Yoshimitsu K, Nishihara Y, Irie H, Aishima S, Taketomi A, et al. Arterial blood supply of hepatocellular carcinoma and histologic grading: radiologic-pathologic correlation. *AJR Am J Roentgenol*. 2008; 190: W28–W34.
34. Nakachi K, Tamai H, Mori Y, Shingaki N, Moribata K, Deguchi H, et al. Prediction of poorly differentiated hepatocellular carcinoma using contrast computed tomography. *Cancer Imaging*. 2014; 14: 7.
35. Cheung TT, Ho CL, Lo CM, Chen S, Chan SC, Chok K S, et al. 11C-acetate and 18F-FDG PET/CT for clinical staging and selection of patients with hepatocellular carcinoma for liver transplantation on the basis of Milan criteria: surgeon's perspective. *J Nucl Med*. 2013; 54: 192–200.
36. Park JW, Kim JH, Kim SK, Kang KW, Choi JI, et al. A prospective evaluation of 18F-FDG and 11C-acetate PET/CT for detection of primary and metastatic hepatocellular carcinoma. *J Nucl Med*. 2008; 49: 1912–1921.
37. Chen J, Wu M, Liu R, Li S, Gao R, Song B. Preoperative evaluation of the histological grade of hepatocellular carcinoma with diffusion-weighted imaging: a meta-analysis. *PLoS One*. 2015; 10: e0117661.
38. Liu C, Xiao XQ, Yan LN, Li B, Jiang L, Wen TF, et al. Value of α -fetoprotein in association with clinicopathological features of hepatocellular carcinoma. *World J Gastroenterol*. 2013; 19: 1811–1819.
39. Oishi K, Itamoto T, Amano H, Fukuda S, Ohdan H, Tashiro H, et al. Clinicopathologic features of poorly differentiated hepatocellular carcinoma. *J Surg Oncol*. 2007; 95: 311–316.
40. Ji SP, Li Q, Dong H. Therapy and prognostic features of primary clear cell carcinoma of the liver. *World J Gastroenterol*. 2010; 16: 764–769.
41. Lai CL, Wu PC, Lam KC, Todd D. Histologic prognostic indicators in hepatocellular carcinoma. *Cancer*. 1979; 44: 1677–1683.
42. Albar JP, De Miguel F, Esbrit P, Miranda R, Fernandez-Flores A, Sarasa JL. Immunohistochemical detection of parathyroid hormone-related protein in a rare variant of hepatic neoplasm (sclerosing hepatic carcinoma). *Hum Pathol*. 1996; 27: 728–731.
43. El-Serag HB, Davila JA. Is fibrolamellar carcinoma different from hepatocellular carcinoma? A US population-based study. *Hepatology*. 2004; 39: 798–803.
44. Torbenson M. Review of the clinicopathologic features of fibrolamellar carcinoma. *Adv Anat. Pathol*. 2007; 14: 217–223.
45. Vecchio FM, Fabiano A, Ghirlanda G, Manna R, Massi G. Fibrolamellar carcinoma of the liver: The malignant counterpart of focal nodular hyperplasia with oncocytic change. *Am J Clin Pathol*. 1984; 81: 521–526.
46. Ross HM, Daniel HD, Vivekanandan P, Kannangai R, Yeh MM, Wu TT. Fibrolamellar carcinomas are positive for CD68. *Mod Pathol*. 2011; 390–395.
47. Allen RA, Lisa JR. Combined liver cell and bile duct carcinoma. *Am J Pathol*. 1949; 25: 647–55.
48. Koh KC, Lee H, Choi MS, Lee JH, Paik SW, Yoo BC, et al. Clinicopathologic features and prognosis of combined hepatocellular cholangiocarcinoma. *Am J Surg*. 2005; 189: 120–125.
49. Taguchi J, Nakashima O, Tanaka M, Hisaka T, Takazawa T, Kojiro M. A clinicopathological study on combined hepatocellular and cholangiocarcinoma. *J. Gastroenterol. Hepatol*. 1996; 11: 758–764.
50. Jarnagin WR, Weber S, Tickoo SK, Koea JB, Obiekwe S, Fong Y. Combined hepatocellular and cholangiocarcinoma: demographic, clinical, and prognostic factors. *Cancer*. 2002; 94: 2040–2046.
51. Yeh MM. Pathology of combined hepatocellular-cholangiocarcinoma. *J Gastroenterol Hepatol*. 2010; 25: 1485–1492.
52. Durnez A, Verslype C, Nevens F, Fevery J, Aerts R, Pirenne J. The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology*. 2006; 49: 138–151.
53. Yano H, Iemura A, Haramaki M, Momosaki S, Ogasawara S, Higaki K, et al. A human combined hepatocellular and cholangiocarcinoma cell line (KMCH-2) that shows the features of hepatocellular carcinoma or cholangiocarcinoma under different growth conditions. *J. Hepatol*. 1996; 24: 413–422.
54. Lee JH, Chung GE, Yu SJ, Hwang SY, Kim JS, Kim HY, et al. Long-term prognosis of combined hepatocellular and cholangiocarcinoma after curative resection comparison with hepatocellular carcinoma and cholangiocarcinoma. *J Clin Gastroenterol*. 2011; 45: 69–75.

55. Liu CL, Fan ST, Lo CM, Ng IO, Lam CM, Poon RT, et al. Hepatic resection for combined hepatocellular and cholangiocarcinoma. *Arch Surg.* 2003; 138: 86–90.
56. Chan AC, Lo CM, Ng IO, Fan ST. Liver transplantation for combined hepatocellular cholangiocarcinoma. *Asian J Surg.* 2007; 30: 143–146.
57. Zuo HQ, Yan LN, Zeng Y, Yang JY, Luo HZ, Liu JW, et al. Clinicopathological characteristics of 15 patients with combined hepatocellular carcinoma and cholangiocarcinoma. *Hepatobiliary Pancreat Dis. Int.* 2007; 6: 161–165.
58. Aoki K, Takayasu K, Kawano T, Muramatsu Y, Moriyama N, Wakao F, et al. Combined hepatocellular carcinoma and cholangiocarcinoma: clinical features and computed tomographic findings. *Hepatology.* 1993; 18: 1090–1095.