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ORIGINAL ARTICLE

Clinicopathological characteristics and liver stem cell marker expression in hepatocellular carcinoma involving bile duct tumor thrombi

Ye-Bin Pang[1,2] · Jian-Hong Zhong[1,3] · Xiao-Ling Luo[2] · Chao Ou[2] · Zhe Guo[1] · Bang-De Xiang[1,3] · Ning-Fu Peng[1,3] · Le-Qun Li[1,3]

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Abstract The aim of this study was to analyze the clinicopathological characteristics and expression of liver stem cell markers of hepatocellular carcinoma (HCC) involving bile duct tumor thrombi (BDTT). A total of 35 patients with HCC and BDTT in a consecutive series of HCC patients who underwent surgical treatment were studied retrospectively and compared with 916 patients without BDTT from the same series. Clinicopathological characteristics, overall survival (OS), and tumor expression of liver stem cell markers CD133, CD90, EpCAM, CK19, VEGF, and C-kit were compared between the two patient groups. Analysis was performed for the entire patient groups as well as for 35 pairs of patients with or without BDTT matched by propensity score. HCC patients with BDTT tended to have smaller tumors than those without BDTT, as well as a higher probability of having poorly differentiated tumor, Child-Pugh class B, liver cirrhosis, and microvascular invasion. Tumor tissue in patients with BDTT showed significantly higher expression rates of all liver stem cell markers examined. OS was significantly lower for patients with BDTT at 1 year (69 vs 84 %), 3 years (37 vs 64 %), and 5 years (20 vs 55 %) ($P<0.001$). Patients with HCC and BDTT show lower OS than patients without BDTT. The higher frequency of liver stem cell marker expression in the presence of BDTT suggests that such stem cells may play a role in the pathogenesis of this form of HCC.

Keywords Hepatocellular carcinoma · Bile duct tumor thrombi · Clinicopathological characteristic · Liver stem cell biomarker · Stem cell

Introduction

Hepatocellular carcinoma (HCC) is a common malignant disease associated with poor prognosis, and its incidence is increasing in many countries, especially the Asia-Pacific region [1]. Despite the continuous development of therapeutic strategies, long-term prognosis remains dismal.

HCC frequently invades the portal vein, hepatic vein, or postcava and forms venous thrombi. However, bile duct tumor thrombi (BDTT) occur in only 1.66–13 % of patients with HCC [2, 3]. HCC patients with BDTT are often misdiagnosed with choledocholithiasis or cholangiocarcinoma due to a lack of understanding of the clinicopathological features of this uncommon form of HCC. Traditionally, BDTT is thought to occur late in HCC when tumor cells invade the bile duct wall, marking a terminal stage in the disease. However, recent studies suggest that BDTT may occur early in HCC, when the primary lesion is still small or even undetectable. Tumor thrombi can detach easily from the bile duct wall. Furthermore, there is no biological evidence of infiltration by tumor cells [4, 5]. The mechanism of BDTT formation remains controversial. A potential clue to BDTT pathogenesis came when

Ye-Bin Pang, Jian-Hong Zhong and Xiao-Ling Luo contributed equally to this work.

✉ Ning-Fu Peng
pengnfu2013@163.com

✉ Le-Qun Li
xitongpingjia@163.com

[1] Hepatobiliary Surgery Department, Affiliated Tumor Hospital of Guangxi Medical University, He Di Rd. #71, Nanning 530021, People's Republic of China

[2] Research Department, Affiliated Tumor Hospital of Guangxi Medical University, Nanning 530021, People's Republic of China

[3] Guangxi Liver Cancer Diagnosis and Treatment Engineering and Technology Research Center, Nanning 530021, People's Republic of China

our group discovered that a handful of tumor cells in BDTT of HCC patients show the characteristics of liver stem cells [6].

To help improve the detection and diagnosis of HCC with BDTT and explore a possible relationship with liver stem cells, we analyzed the clinicopathological characteristics of patients with HCC and BDTT who underwent hepatic resection at a large medical center in China. We also examined stem cell marker expression in tumor tissues. We compared the results with those from HCC patients without BDTT from the same hospital population.

Materials and methods

Patients

The study protocol was approved by the Research Ethics Committee of the Affiliated Tumor Hospital of Guangxi Medical University, which waived the requirement for informed consent for this retrospective study. Patients were considered for inclusion if they were diagnosed with HCC based on histopathology of surgical samples taken between 1 January 2000 and 31 December 2012 at the Affiliated Tumor Hospital of Guangxi Medical University. Patients within this group in whom BDTT were detected by ultrasonography, computed tomography, or magnetic resonance imaging before, during, or after surgery were classified as HCC patients with BDTT for the present study. The remaining patients were classified as HCC patients without BDTT and served as the control group in the present study.

Medical records and paraffin-embedded tumor specimens were obtained for patients with and without BDTT. All patients were treated surgically as described [7, 8], without prior chemo- or radiotherapy. Patients were followed up every 1–2 months for the first 6 months after surgery, and every 3–6 months thereafter. At each follow-up visit, clinical and laboratory tests were performed, including chest X-ray and abdominal ultrasound or computed tomography. All patients were followed up until death or January 2015.

Clinicopathological definitions

Tumor size was determined based on preoperative imaging and confirmed by postoperative histology examination [9, 10]. Microvascular invasion was defined as the presence of a tumor thrombus based on microscopy. Liver cirrhosis was determined based on postoperative pathology examination.

Immunohistochemistry

Tumor tissue sections were immunostained using the Power Vision Two-Step Kit (ZSG, Beijing, China) according to the manufacturer's instructions. Monoclonal antibodies against the following liver stem cell markers were used: CD133 (1:200; Miltenyi Biotec, Germany), CD90 (1:100; Abcam, Cambridge, UK), EpCAM (1:200; Abcam), C-kit (1:200; Maxim, Fuzhou, China), CK-19 (1:200; Maxim), and VEGF (1:200; Maxim). Stained sections were analyzed independently by two investigators blinded to sample details. These investigators graded the percentage of positive cells (PP) on a 5-point scale: 0 (no positive cells), 1 (positive cells ≤25 %), 2 (positive cells 26–50 %), 3 (positive cells 51–75 %), and 4 (positive cells >75 %). They also graded staining intensity (SI) on a 3-point scale: 0 (no staining), 1 (mild), 2 (moderate), and 3 (strong). The two scores for PP and SI were multiplied together to give an overall score for each section ranging from 0 to 12. Overall scores of 0–3 were considered negative; scores of 4–12, positive.

Propensity score analysis

HCC patients with and without BDTT can differ in numerous characteristics, some of which may not be related to BDTT, which increases the risk of bias in comparisons between the two patient groups. To compensate for baseline differences, we performed propensity score matching [11]. The propensity model contained the following clinical variables: gender, age, Child-Pugh class, serum bilirubin, albumin, α-fetoprotein (AFP), prothrombin time, hepatitis B surface antigen positivity, hepatitis C antibody positivity, tumor size, tumor capsule, lymphatic metastasis, liver cirrhosis, microvascular invasion, portal vein invasion, and tumor differentiation. These variables were assembled into a logistic regression model, which was used to generate propensity scores for each patient along a continuous range from 0 to 1. The one-to-one nearest-neighbor matching [12] was used to pair patients with or without BDTT.

Statistical analysis

Statistical analysis was conducted using SPSS 16.0 (IBM, Chicago, IL, USA) and a significance threshold of $P<0.05$. Continuous data were reported as mean± standard error. Immunohistochemical differences between patient groups were assessed for significance using the chi-squared test or Fisher's exact test, as appropriate. Overall survival (OS) was evaluated using the Kaplan-Meier method, and intergroup differences were assessed using the log-rank test. Possible associations between clinicopathological characteristics and expression of liver stem cell biomarkers were assessed using Kendall's tau-b correlation. The statistical data were managed by a biostatistician.

Results

Clinicopathological characteristics

During the study period, 951 patients with HCC were treated by surgical resection at the Affiliated Tumor Hospital, of whom 35 were diagnosed with BDTT (Table 1). Comparison of these 35 patients with the remaining 916 without BDTT showed that BDTT was associated with smaller tumor size ($P<0.001$) and higher frequency of liver cirrhosis, Child-Pugh class B, and microvascular invasion ($P<0.05$). The two groups did not differ significantly in gender; age; levels of serum bilirubin, albumin, or AFP; prothrombin time; or frequencies of hepatitis B surface antigen positivity, hepatitis C antibody positivity, tumor capsule, lymphatic metastasis, or portal vein invasion. As expected, no significant clinicopathological differences were found between patients with or without BDTT among the 35 propensity score-matched pairs (Table 1).

Overall survival

Patients were followed up for a median of 96 months (range, 1–121). Among the 35 patients with BDTT, one died of perioperative liver failure, ten died of tumor recurrence, and five experienced recurrence in the remnant liver. OS among patients with BDTT was 69 % at 1 year, 37 % at 3 years, and 20 % at 5 years. The corresponding rates for 916 HCC patients without BDTT were 84, 64, and 55 % [9]. The log-rank test showed that OS was significantly better among patients without BDTT ($P<0.001$; Fig. 1). Similar results were obtained among the 35 pairs of propensity score-matched patients ($P<0.001$, Fig. 2); OS in this subset of patients without BDTT was 83 % at 1 year, 66 % at 3 years, and 62 % at 5 years.

Expression of liver stem cell markers

In tumor tissue from HCC patients with or without BDTT, staining for CD133, CD90, and C-kit was observed primarily in cell membrane and cytoplasm, while staining for CK19, VEGF, and EpCAM was observed in cell membrane (Fig. 3). Only faint staining for the six markers was observed in adjacent non-tumor tissue (data not shown). Comparison of tumor sections from patients with and without BDTT from among the 35 propensity

Table 1 Comparison of demographic and clinicopathological characteristics of hepatocellular carcinoma patients with or without bile duct tumor thrombi

		Before propensity matching		After propensity matching	
Characteristic	HCC with BDTT (n=35)	HCC without BDTT (n=916)	P^b	HCC without BDTT (n=35)	P^c
Gender, M/F	30/5	824/92	0.673	30/5	1.000
Age, year	50.1±10.2	51.8±1.4	0.235	50.8±9.7	0.752
Child-Pugh A/B, *n* (%)	25 (71)/10 (29)	778 (85)/138 (15)	0.024	24 (69)/11 (31)	0.537
TBIL, mg/dL	1.9±0.1	1.3±0.8	0.463	1.6±0.3	0.779
ALB, g/dL	3.2±0.7	3.9±0.9	0.745	3.5±0.5	0.865
PT, s	12.8±2.3	13.2±5.4	0.674	12.1±3.4	0.731
Serum AFP, *n* (%)					
≥400 ng/mL	16 (46)	439 (48)	0.758	14 (40)	0.686
<400 ng/mL	19 (54)	477 (52)		21 (60)	
HBsAg, *n* (%)	31 (89)	852 (93)	0.166	30 (85)	0.406
HCV antibody, *n* (%)	2 (5)	18 (2)	0.065	1 (3)	0.873
Lymphatic metastasis, *n* (%)	4 (11)	55 (6)	0.095	2 (6)	0.605
Tumor size, cm	3.76±1.01	8.02±1.63	< 0.001	4.08±1.01	0.691
Intact tumor capsule, *n* (%)	12 (34)	275 (30)	0.853	13 (37)	0.913
Liver cirrhosis, *n* (%)	32 (91)	659 (72)	0.033	30 (86)	0.728
Microvascular invasion, *n* (%)	15 (43)	247 (27)	0.032	15 (43)	1.000
Portal vein invasion, *n* (%)	9 (26)	219 (24)	0.650	8 (23)	0.784
Poor differentiation[a], *n* (%)	15 (43)	183 (20)	< 0.001	13 (37)	0.384

Abbreviations: *AFP* α-fetoprotein, *ALB* albumin level, *BDTT* bile duct tumor thrombi, *HBsAg* hepatitis B virus surface antigen, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *PT* prothrombin time, *TBIL* total bilirubin level

[a] Based on Edmondson-Steiner grading

[b] Comparing all 35 HCC patients with BDTT to all 916 HCC patients without BDTT

[c] Comparing 35 HCC patients with BDTT matched to 35 patients without BDTT, based on propensity scoring

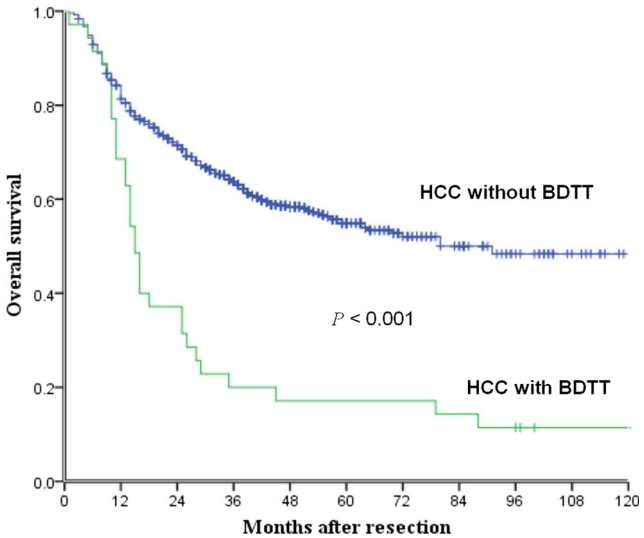

Fig. 1 Overall survival curves for 916 patients with hepatocellular carcinoma (HCC) without bile duct tumor thrombi (BDTT) and for 35 patients with HCC and BDTT

score-matched pairs revealed significantly higher expression rates for all six markers in BDTT samples (Table 2). All six markers were co-expressed in 23 patients with BDTT (65.7 %), but in only two patients without BDTT (5.7 %; $P<0.001$). Expression of CD133, CD90, EpCAM, and CK19 negatively correlated with tumor differentiation (Table 3): expression was higher in poorly differentiated tumors than in moderately or well differentiated ones. All BDTT were found detached from the bile duct wall in pathological sections, and none of the 35 propensity score-matched patients with BDTT showed macro- or microscopic evidence of bile duct wall infiltration by tumor cells.

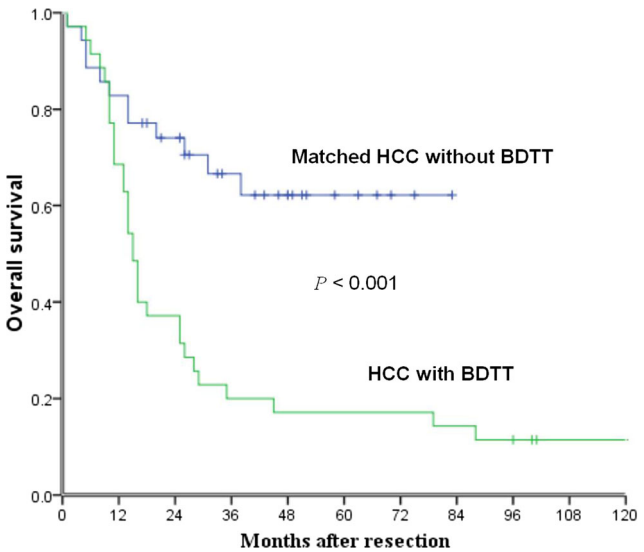

Fig. 2 Overall survival curves for 35 patients with hepatocellular carcinoma (HCC) and bile duct tumor thrombi (BDTT) and 35 HCC patients without BDTT, matched based on propensity scoring

Discussion

The present retrospective study may help clarify the clinicopathological picture of HCC with BDTT, which in turn may improve diagnosis and management. Incidence of BDTT in our cohort of HCC patients was 3.68 % (35/951), and the accuracy of initial diagnosis was 51.43 % (18/35; data not shown), similar to the 52.94 % reported in another Chinese study [13]. Patients with this kind of tumor often present with jaundice and complain of cholecystalgia, shiver, and fever, leading to misdiagnosis as cholangiocarcinoma or choledocholithiasis. Based on our clinical experience, we propose that elevated serum AFP levels together with imaging results indicating a liver mass and cholangiectasis are strong diagnostic indicators of HCC with BDTT. Of the 35 patients with BDTT in our study, 19 (54.3 %) had a primary tumor smaller than 5 cm and five had no detectable primary tumor. This is consistent with recent studies suggesting that, contrary to the widespread opinion that BDTT occur in advanced HCC, the condition can frequently occur when the primary tumor is very small [5, 13].

Among our 35 patients with BDTT, 29 had macroscopic BDTT detected during surgery, while the remaining six had microscopic BDTT detected in postoperative histological analysis of primary tumor sections. None of the 35 patients showed evidence of bile duct wall infiltration by tumor cells. These results contrast with proposed mechanisms of tumor thrombus formation that begin with HCC invasion into the biliary tree [13, 14]. Consistent with our study in Chinese patients, a study of 17 Japanese HCC patients with BDTT found no evidence of microscopic infiltration of tumor cells into the bile duct wall [3]. In our patients, tumor thrombi were fleshy, fragile, and grayish-white, and they did not adhere tightly to the bile duct wall. As a result, they may separate easily from the duct wall. These findings are consistent with results from studies in Chinese [5] and Caucasian [15] HCC patients with BDTT.

A key motivation for clarifying the clinicopathological characteristics of HCC with BDTT is to guide treatment, since it remains unclear whether patients with this form of HCC benefit from early, active surgery. Studies of Chinese [16] and Japanese [17] patients suggested resectability rates of 10–23 % for this form of HCC, as well as better OS for patients without BDTT. In our cohort, all 35 patients with BDTT were judged to be resectable and consented to that procedure; OS was significantly better among those without BDTT. These results from three studies contrast with other studies in Japan [3, 4] reporting no difference in OS between patients with and without BDTT. We suggest that resection of carefully

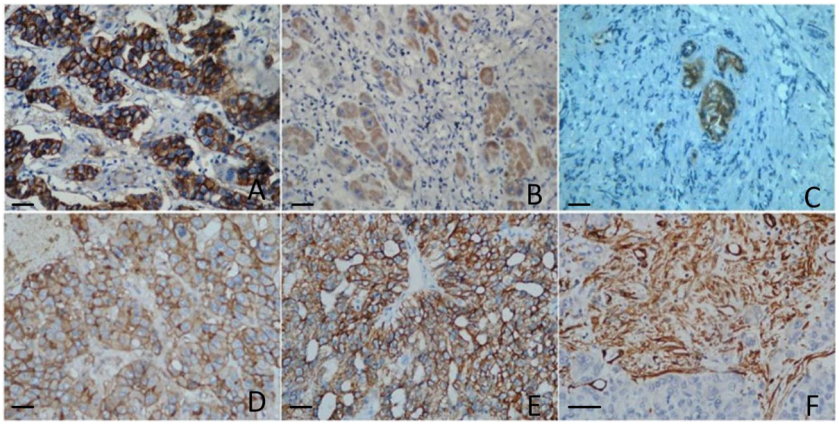

Fig. 3 Power Vision Two-Step staining to detect expression of liver stem cell markers in hepatocellular carcinoma patients with bile duct tumor thrombi. **a** EpCAM. **b** CD133. **c** CK19. **d** CD90. **e** C-kit. **f** VEGF. *Bars*, 25 μm (**a**, **b**, **c**, **d**, **e**) and 50 μm (**f**)

selected HCC patients with BDTT can still result in reasonably good OS. In fact, subgroup analysis by treatment type suggests good OS even with complex interventions: 11 of our patients with BDTT underwent resection of tumor thrombi and bile duct and showed a median OS of 14 months (range, 1–25); 12 patients underwent primary tumor lesion resection, and median OS was 34 months (range, 14–62); 3 patients underwent hepatectomy with thrombectomy and T-tube drainage, and median OS was 51 months (range, 30–98); and the remaining 9 patients underwent hepatectomy, and median OS was 73 months (range, 47–121).

We found that the liver stem cell markers CD133, CD90, EpCAM, CK19, VEGF, and C-kit were expressed at higher levels in primary tumors from HCC patients with BDTT than in tumors from patients without BDTT. This confirms and extends our previous report that a handful of tumor cells in BDTT of HCC patients show the characteristics of liver stem cells [6]. This suggests that liver stem cells may help drive pathogenesis of HCC involving BDTT. This would be consistent with mounting evidence that HCC arises when the differentiation of normal liver stem cells is blocked, causing the stem cells to mutate into tumor cells [18]. Future studies should directly examine the possible involvement of liver stem cells in BDTT and aim to identify the source of these cells. Evidence suggests that the canals of Hering, also known as the terminal bile ductular system, are the most likely origin of stem/progenitor cells in adult liver [19–21]. In previous work, we found that intra-hepatic biliary epithelial cells expressed liver stem cell markers [6], consistent with the idea that the canals of Hering contain liver stem cells. In the present study, we found that expression of CD133, CD90, EpCAM, and CK19 correlated negatively with tumor differentiation. It may be that these aggressive, poorly differentiated tumor cells survive in the toxic microenvironment around the bile duct, where necessary growth factors and nutrients are scarce; liver cancer stem cells are presumed to live in such unfavorable environments [22]. However, since the adjacent tumor tissue and tumor stroma region also play a significant role in the tumor cell migration and that the invasion of HCC into the biliary tree ultimately leads to the formation of BDTT, it would enrich the clinical significance if further studies also investigate and analyze these liver stem cell markers in the adjacent tumor tissue.

Our results, together with those from previous studies, lead us to speculate that liver stem cells in the canals of Hering help drive pathogenesis of HCC involving BDTT. This may explain why BDTT can develop when the primary HCC tumor is still small or even undetectable, as well as why most patients with BDTT show no bile duct wall invasion by tumor cells. Future

Table 2 Expression of liver stem cell markers in propensity score-matched hepatocellular carcinoma patients with or without bile duct tumor thrombi

Liver stem cell marker	Patients showing expression, *n* (%)		
	HCC with BDTT (*n*=35)	HCC without BDTT (*n*=35)	*P*
CD133	28 (80.0)	19 (54.3)	0.032
CD90	29 (82.9)	20 (57.1)	0.019
EpCAM	27 (77.1)	19 (54.3)	0.044
CK-19	25 (71.4)	12 (34.3)	0.002
C-kit	28 (80.0)	18 (51.4)	0.025
VEGF	30 (85.7)	23 (65.7)	0.035

Table 3 Kendall's tau-b analysis to assess the strength of associations between expression of liver stem cell markers and tumor differentiation in propensity score-matched hepatocellular carcinoma patients with or without bile duct tumor thrombi

Liver stem cell marker	*R*	*P*
CD133	0.369	0.01
CD90	0.596	< 0.001
EpCAM	0.396	0.006
CK-19	0.376	0.009
C-kit	–	0.175
VEGF	–	0.107

studies in the laboratory as well as large, multi-center clinical investigations are needed in order to elucidate whether and how liver stem cell transformation relates to HCC and BDTT.

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Compliance with ethical standards

Conflicts of interest None.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65:87–108.
2. Ueda M, Takeuchi T, Takayasu T, Takahashi K, Okamoto S, Tanaka A, et al. Classification and surgical treatment of hepatocellular carcinoma(HCC) with bile duct thrombi. Hepatogstroenterology. 1994;41:349–54.
3. Satoh S, Ikai I, Honda G, Okabe H, Takeyama O, Yamamoto Y, et al. Clinicopathologic evaluation of hepatocellular carcinoma with bile duct thrombi. Surgery. 2000;128:779–83.
4. Shiomi M, Kamiya J, Nagino M, Uesaka K, Sano T, Hayakawa N, et al. Hepatocellular carcinoma with biliary tumor thrombi: Aggressive operative approach after appropriate preoperative management. Surgery. 2001;129:692–8.
5. Peng BG, Liang LJ, Li SQ, Zhou F, Hua YP, Luo SM. Surgical treatment of hepatocellular carcinoma with bile duct tumor thrombi. World J Gastroenterol. 2005;11:3966–9.
6. Peng N, Li L, Cai X, Tan S, Wu T. Liver stem/progenitor cells in the canals of Hering: cellular origin of hepatocellular carcinoma with bile duct tumor thrombi. Stem Cell Rev. 2010;6:579–84.
7. Zhong JH, You XM, Lu SD, Wang YY, Xiang BD, Ma L, et al. Historical comparison of overall survival after hepatic resection for patients with large and/or multinodular hepatocellular carcinoma. Medicine (Baltimore). 2015;94, e1426.
8. Zhong JH, Xiang BD, Gong WF, Ke Y, Mo QG, Ma L, et al. Comparison of long-term survival of patients with BCLC stage B hepatocellular carcinoma after liver resection or transarterial chemoembolization. PLoS One. 2013;8, e68193.
9. Zhong JH, Ke Y, Gong WF, Xiang BD, Ma L, Ye XP, et al. Hepatic resection associated with good survival for selected patients with intermediate and advanced-stage hepatocellular carcinoma. Ann Surg. 2014;260:329–40.
10. Torzilli G, Belghiti J, Kokudo N, Takayama T, Capussotti L, Nuzzo G, et al. A snapshot of the effective indications and results of surgery for hepatocellular carcinoma in tertiary referral centers: is it adherent to the EASL/AASLD recommendations?: an observational study of the HCC East-west study group. Ann Surg. 2013;257:929–37.
11. D'Aqostino Jr R. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. Stat Med. 1998;17:2265–81.
12. Hung HH, Chiou YY, Hsia CY, Su CW, Chou YH, Chiang JH, et al. Survival rates are comparable after radiofrequency ablation or surgery in patients with small hepatocellular carcinomas. Clin Gastroenterol Hepatol. 2011;9:79–86.
13. Qin LX, Ma ZC, Wu ZQ, Fan J, Zhou XD, Sun HC, et al. Diagnosis and surgical treatments of hepatocellular carcinoma with tumor thrombosis in bile duct: experience of 34 patients. World J Gastroenterol. 2004;10:1397–401.
14. Liu Q, Chen J, Li H, Liang B, Zhang L, Hu T. Hepatocellular carcinoma with bile duct tumor thrombi: correlation of magnetic resonance imaging features to histopathologic manifestations. Eur J Radiol. 2010;76:103–9.
15. Edmondson H, Steiner P. Primary carcinoma of the liver—a study of 100 cases among 48900 necropsies. Cancer. 1954;7:462–503.
16. Xiangji L, Weifeng T, Bin Y, Chen L, Xiaoqing J, Baihe Z, et al. Surgery of hepatocellular carcinoma complicated with cancer thrombi in bile duct: efficacy for criteria for different therapy modalities. Langenbecks Arch Surg. 2009;394:1033–9.
17. Maruyama H, Yoshida H, Hirakata A, Matsutani T, Yokoyama T, Suzuki S, et al. Surgical treatment of a patient with diaphragmatic invasion by a ruptured hepatocellular carcinoma with biliary and portal venous tumor thrombi. J Nippon Med Sch. 2012;79:147–52.
18. Sell S. Heterogeneity and plasticity of hepatocyte lineage cells. Hepatology. 2001;33:738–50.
19. Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, et al. The canals of Hering and hepatic stem cells in humans. Hepatology. 1999;30:1425–33.
20. De Alwis N, Hudson G, Burt AD, Day CP, Chinnery PF. Human liver stem cells originate from the canals of Hering. Hepatology. 2009;50:992–3.
21. Fellous TG, Islam S, Tadrous PJ, Elia G, Kocher HM, Bhattacharya S, et al. Locating the stem cell niche and tracing hepatocyte lineages in human liver. Hepatology. 2009;49:1655–63.
22. Mishra L, Banker T, Murray J, Byers S, Thenappan A, He AR, et al. Liver stem cells and hepatocellular carcinoma. Hepatology. 2009;49:318–29.