RESEARCH ARTICLE

Clinicopathologic Characteristics and Prognoses for Multicentric Occurrence and Intrahepatic Metastasis in Synchronous Multinodular Hepatocellular Carcinoma Patients

Shi-Lai Li[1], Ming Su[1], Tao Peng[1], Kai-Yin Xiao[1], Li-Ming Shang[1], Bang-Hao Xu[1], Zhi-Xiong Su[1], Xin-Ping Ye[1], Ning Peng[1], Quan-Lin Qin[1], De-Feng Chen[1], Jie Chen[2], Le-Qun Li[2]*

Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and the outcomes for patients are still poor. It is important to determine the original type of synchronous multinodular HCC for preoperative assessment and the choice of treatment therapy as well as for the prediction of prognosis after treatment. Aims: To analyze clinicopathologic characteristics and prognoses in patients with multicentric occurrence (MO) and intrahepatic metastasis (IM) of synchronous multinodular hepatocellular carcinoma (HCC). Methods: The study group comprised 42 multinodular HCC patients with a total of 112 nodules. The control group comprised 20 HCC patients with 16 single nodular HCC cases and 4 HCC cases with a portal vein tumor emboli. The mitochondrial DNA (mtDNA) D-loop region was sequenced, and the patients of the study group were categorized as MO or IM based on the sequence variations. Univariate and multivariate analyses were used to determine the important clinicopathologic characteristics in the two groups. Results: In the study group, 20 cases were categorized as MO, and 22 as IM, whereas all 20 cases in the control group were characterized as IM. Several factors significantly differed between the IM and MO patients, including hepatitis B e antigen (HBeAg), cumulative tumor size, tumor nodule location, cirrhosis, portal vein and/or microvascular tumor embolus and the histological grade of the primary nodule. Multivariate analysis further demonstrated that cirrhosis and portal vein and/or microvascular tumor thrombus were independent factors differentiating between IM and MO patients. The tumor-free survival time of the MO subjects was significantly longer than that of the IM subjects (25.7±4.8 months vs. 8.9±3.1 months, p=0.017). Similarly, the overall survival time of the MO subjects was longer (31.6±5.3 months vs. 15.4±3.4 months, p=0.024). The multivariate analysis further demonstrated that the original type (p=0.035) and Child-Pugh grade (p<0.001) were independent predictors of tumor-free survival time. Cirrhosis (p=0.011), original type (p=0.034) and Child-Pugh grade (p<0.001) were independent predictors of overall survival time. Conclusions: HBeAg, cumulative tumor size, tumor nodule location, cirrhosis, portal vein and/or microvascular tumor embolus and histological grade of the primary nodule are important factors for differentiating IM and MO. MO HCC patients might have a favorable outcome compared with IM patients.

Keywords: Characteristic - prognosis - multicentric occurrence - intrahepatic metastasis - hepatocellular carcinoma

Asian Pacific J Cancer Prev, **14 (1)**, 217-223

Introduction

Hepatocellular carcinoma (HCC), which is the fifth most common cancer and the third leading cause of cancer-related death worldwide, is a global health problem. The estimated annual number of cases exceeds 600 000, and over 55% occur in China (Parkin et al., 2005). Many types of treatment are performed for HCC, but curative resection is still the first choice. The largest challenge for HCC treatment is recurrence after curative resection therapy (Perry et al., 2007). The five-year survival rate was lower than 5% in developing countries in 2002 (Parkin et al., 2005). As reported, recurrent HCC has two origins: multicentric occurrence (MO) and intrahepatic metastasis (IM) (Yamamoto et al., 1999). The MO recurrence of HCC is new and different from the primary lesion, but for IM, the recurrence is derived from the primary tumor. Patients with MO have better outcomes than those with IM (Yasui et al., 1997; Huang et al., 2012). Many studies (Oda et al., 1992; Liver Cancer Study Group of Japan, 1997; Yamamoto et al., 1999; Chen et al., 2000; Nomoto et al., 2002; Okamoto et al., 2006; Nomoto et al., 2007;

[1]*Department of Hepatobiliary Surgery, the First Affiliated Hospital of Guangxi Medical University,* [2]*Department of Hepatobiliary Surgery, Tumor Hospital Affiliated of Guangxi Medical University, Nanning, China *For correspondence: lilequn2011@yahoo.cn*

Li et al., 2008) have examined the differentiation of the origins of recurrent HCC, also known as metachronous multiple HCC, because the original type is important for the preoperative assessment, determining the treatment modality and predicting patient prognosis. The same problem also appears in synchronous multinodular HCC patients, but few studies have examined this population. In this study, we identify the cell clonal origin of synchronous multinodular HCC by analyzing variations in the mitochondrial DNA (mtDNA) D-loop region, and we also compare the clinicopathologic characteristics and prognoses between MO and IM patients.

Materials and Methods

Clinical HCC Samples

The study group was composed of 42 multinodular HCC patients with a total of 112 HCC nodules who were hospitalized for the radical resection of HCC in the Department of Hepatobiliary Surgery of the First Affiliated Hospital of Guangxi Medical University from April 2004 to August 2007. The study comprised 36 men and 6 women. The median age for the 42 patients was 42.5 years (range, 23–64 years). The control group was composed of 20 HCC patients (40 samples) who were hospitalized during the same period, and it consisted of two sub-groups: control group I and control group II. Control group I consisted of 16 single nodular HCC patients who each had 2 samples of non-consecutive tumor tissues, and control group II consisted of 4 HCC patients with a portal vein tumor embolus whose tumor tissues and portal vein tumor emboli were collected simultaneously. All samples were confirmed using histopathology. Informed consent was obtained from each participant before sample collection, and this study was approved by the medical ethics committee of the First Affiliated Hospital of Guangxi Medical University.

DNA Extraction

All samples were immediately fresh-frozen after resection and stored at −80°C. Serial 4 μm sections were cut with a microtome and stained with H&E for histological examination. The remaining tissue was used for DNA extraction according to the specifications of the DNA extraction kit (TIANamp Genomic DNA Kit, China).

Amplification of the Mitochondrial D-loop Fragment

MtDNA fragments of 1 528 bp in length containing 1 122 bp of the D-loop region were amplified using the following primers: F, 5'ATTCTAACCTGAATCGGAGG3' (forward); and R, 5'GATGCTTGCATGTGTAATCT3' (reverse) (Shanghai Sangon Biological Engineering Technology And Service Co., Ltd, China). Each DNA sample (50 ng) was amplified using polymerase chain reaction (PCR). PCR (initial incubation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min) was performed in a final volume of 50 μl with the PCR kit Taq Hot-Start Version (TaKaRa, Japan).

Purification and Direct Sequencing of the D-loop Region of MtDNA

The PCR products were purified with a PCR purification kit and sequenced with an Applied Biosystems DNA sequence (ABI 3730XL, USA). The primers used for sequencing were as follows: F, 5'ATTCTAACCTGAATCGGAGG3' (forward) and R, 5'GATGCTTGCATGTGTAATCT3' (reverse). Because many structures contain poly C in the mtDNA D-Loop region, two additional primers were used (F2: 5'AATACTTGACCACCTGTAG'3 and F3: 5'CCTATGTCGCAGTATCTGTC'3) to sequence the entire mtDNA D-Loop region. The sequenced regions were overlapped by multiple primers. All mutations were confirmed by a repeated analysis of the mtDNA extracted from the tissue samples. All these procedures were performed by Beijing Sunbiotech Co., Ltd. (Beijing, China).

Analyzing the Sequence Results

The sequences of the tumor tissues were compared with the standard mtDNA sequence according to GenBank using DNAstar software. And then the variations of the sequences were considered as polymorphisms or mutations according to the mtDNA database (http://www.mitomap.org/).

Determining Tumor Clonality

Tumor clonality was determined based on the variation status of the mtDNA D-Loop sequences of the multinodular lesions. Basically, when the variation status of the mtDNA D-Loop sequences was the same in different nodules from the same patient, we assumed that the tumor nodules were related to each other, i.e., IM. Similarly, when the variation status of the mtDNA D-Loop sequences differed between nodules from the same patient, we assumed that each different nodule was an independent lesion, i.e., MO.

Follow-up

All patients were followed up at the outpatient clinic every 3 months, and their serum alpha-fetoprotein levels were measured. Hepatic ultrasonography was performed every 2-4 months from the date of the initial treatment up to July 2012 or death. When a patient was suspected of having a recurrence, further evaluations were made using abdominal computed tomography (CT) or Magnetic Resonance Imaging (MRI) to confirm the diagnosis. The defined end point was death.

Statistics

SPSS for Windows 13.0 statistical software was used to perform the analyses. The clinicopathologic factors were compared between the IM and MO patients using the chi-square test and Fisher's exact test. For the multivariate analysis, a stepwise logistic regression model was used to identify the most important factors differentiating between the IM and MO patients. The tumor-free survival (recurrence) and overall survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test. The factors associated with recurrence or overall survival after hepatic resection were analyzed in a multivariate analysis in Cox's proportional hazards

Table 1. Univariate Analysis of the Clinicopathologic Factors Associated with MO and IM Patients

Clinicopathologic variables	MO group (n=20)	IM group (n=22)	χ^2 value	p value
Gender (male/female)	19:1	17:5	a	0.187
Age (≤42.5yr vs. >42.5yr)	8:12	13:9	1.527	0.217
Alcohol abuse (abuse/non-abuse)	2:18	7:15	a	0.135
Platelet Count (<100×10^9/L vs. ≥100×10^9/L)	0:20	0:20	-	-
HBsAg (positive/negative)	18:2	21:1	a	0.598
HCVAb (positive/negative)	0:20	0:22	-	-
HBeAg (positive/negative)	8:12	1:21	a	0.008
HBeAb (positive/negative)	10:10	16:6	2.295	0.130
Tumor size of the primary nodule (≤5 cm vs. >5 cm)	9:11	6:16	1.434	0.231
Cumulative tumor size (≤7 cm vs.>7 cm)	10:10	4:18	4.773	0.029
Tumor number (<3 vs.≥3)	12:8	14:8	0.059	0.808
Tumor nodule location (left hepatic lobe/right hepatic lobe/both lobes)	1:13:6	7:13:2	6.419	0.040
Cirrhosis (present/absent)	18:2	12:10	6.453	0.011
Primary nodule capsule (present/absent)	9:11	5:17	2.339	0.126
AFP (≥20μg/L vs. <20μg/L)	12:8	17:5	1.462	0.227
Portal vein tumor thrombus and/or microvascular tumor thrombus (present/absent)	2:18	9:13	5.177	0.023
Histological grade of the primary nodule (well/moderate/poor)	1:17:2	3:10:9	7.190	0.027
Child-Pugh grade (A/B)	8:12	10:12	0.127	0.721

Chi-square test; a, Fisher's exact test; MO, multicentric occurrence; IM, intrahepatic metastasis; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; HBeAg, hepatitis B e antigen; HBeAb, hepatitis B e antibody; AFP, alpha fetal protein

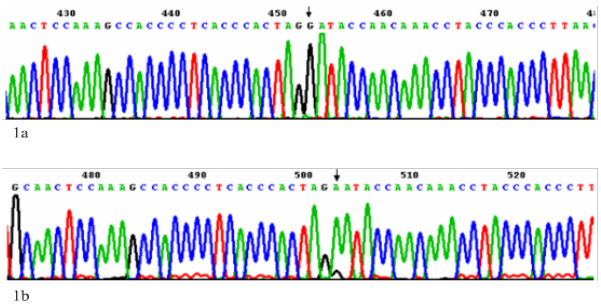

Figure 1. A Case with 2 Tumor Nodules and Different Variations in the mtDNA D-Loop Sequences. The variations were NT453G for 1a, and NT503A for 1b, which corresponded to NT16274G in GenBank. The case was categorized as MO

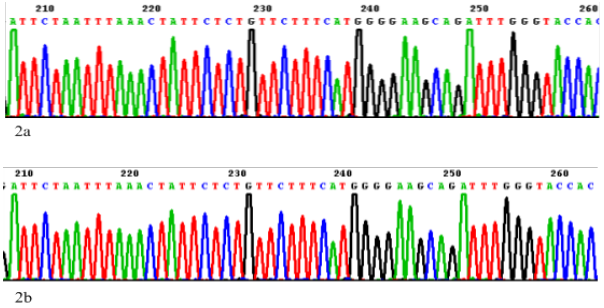

Figure 2. A Case with 2 Tumor Nodules and Identical 2a and 2b mtDNA D-Loop Sequences. The case was categorized as IM

regression model. A p value <0.05 was considered significant.

In the multivariate analysis, Cox's proportional hazards regression model was used to explore the potential factors impacting the tumor-free time (recurrence time) and the overall survival time. Because there were many clinicopathologic factors and only 37 cases, the univariate Kaplan-Meier analysis (differences comparison using a log-rank test) was used to substantially reduce the number of study variables. The factors with p≤0.1 were used in the multivariate analysis. A stepwise regression analysis model (Forward LR) was used to identify the factors differentiating between the two groups. Those with a standard p value of ≤0.05 were entered into the equation, and those with a p value of >0.1 were removed.

Results

Variations of the MtDNA D-Loop Region

Of the 42 patients with a total of 112 HCC nodules in the study group, there were 113 points with nucleotide variations. Of the variations, there were 15 point mutations, 9 insertions, 16 deletions, and 98 polymorphisms (including some insertions and deletions according to the mtDNA database). The variation rate was 11.7% (131/1 122). According to the sequence analysis of the mtDNA D-Loop region, 20 patients had variations in the mtDNA D-Loop sequence between nodules, and they were characterized as MO patients (47.6%, 20/42) (Figure 1). Conversely, 22 patients had the same mtDNA D-Loop sequence in all of their nodules, and they were characterized as IM patients (Figure 2).

To test the feasibility of the assay using the mtDNA D-Loop variations for the determination of the cell clonal origin, 16 single nodular HCC cases and 4 HCC cases with a portal vein tumor embolus were studied as controls. Of the 16 single nodular HCC cases, 2 samples of non-consecutive tumor tissues from the same patient had identical mtDNA D-Loop variations. Similarly, the mtDNA D-Loop variations were identical between the tumor tissue and the portal vein tumor embolus within each patient. As a result, they were all characterized as IM patients.

Clinicopathologic Characteristics in MO and IM Patients

To identify factors with the potential to differentiate MO from IM, the patient clinicopathological characteristics were analyzed. Hepatitis B e antigen (HBeAg), cumulative

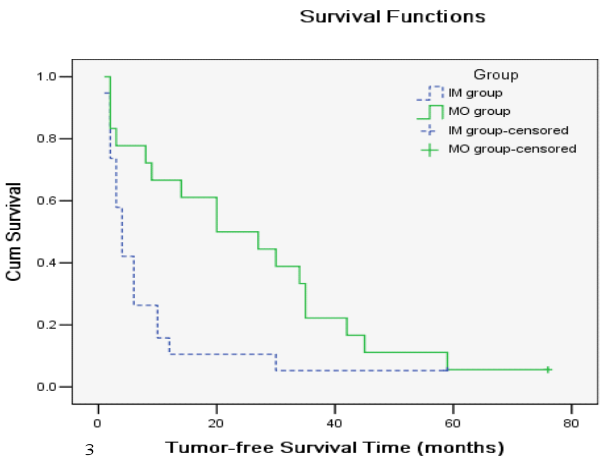

Figure 3. Tumor-free Survival Time for the MO Group and the IM Group. MO group vs. IM group: 25.7±4.8 months vs. 8.9±3.1 months. p=0.017

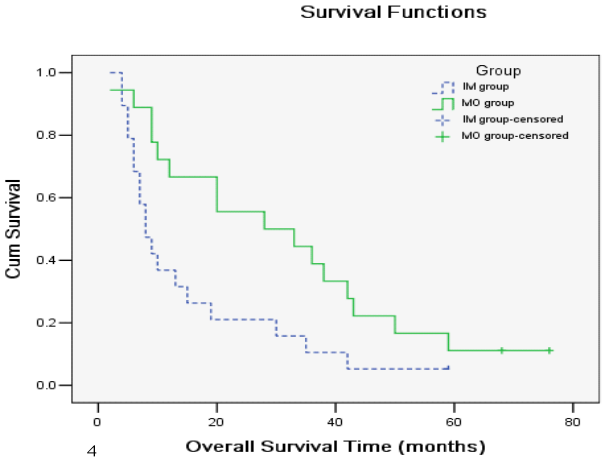

Figure 4. Overall Survival Time for the MO Group and the IM Group. MO group vs. IM group: 31.6±5.3 months vs. 15.4±3.4 months. p=0.024

Table 2. Factors Differentiating Between the Mo Group and the Im Group Identified by A Multivariate Analysis using a Stepwise Logistic Regression Model

Clinicopathologic variables	B	S.E.	Exp(B)	95.0%C.I.for EXP(B)	p value
HBeAg	2.373	1.215	10.735	0.991 - 116.231	0.051
Cirrhosis	2.202	0.990	9.044	1.300 - 62.939	0.026
Portal vein tumor thrombus and/or microvascular tumor thrombus	-2.069	1.006	0.126	0.018 - 0.906	0.040

The variables with p≤0.1 in the univariate analysis were entered in the multivariate analysis. A stepwise logistic regression model (Forward LR) was used with the standard p value ≤ 0.05 entered into the equation, while variables with a p value of > 0.1 were removed

Table 3. Multivariate Analysis of the Clinicopathologic Factors using Cox's Proportional Hazards Regression Model for the Tumor-Free Survival Time and Overall Survival Time

Clinicopathologic variables	B	S.E.	Exp(B)	95.0%C.I.for EXP(B)	p value
Tumor-free survival time					
Cirrhosis	-0.897	0.468	0.408	0.163 - 1.021	0.055
Original type	-0.924	0.439	0.397	0.168 - 0.939	0.035
Child-Pugh grade	1.844	0.440	6.324	2.668 - 14.986	<0.001
Overall survival time					
Cirrhosis	-1.222	0.481	0.295	0.115 - 0.757	0.011
Original type	-0.906	0.427	0.404	0.175 - 0.933	0.034
Child-Pugh grade	2.378	0.496	10.786	4.084 - 28.486	<0.001

Variables with p≤ 0.1 in the univariate analysis entered the multivariate analysis. A stepwise Cox's proportional hazards regression model (Forward LR) was used with the standard p value ≤ 0.05 entered into the equation, while variables with a p value > 0.1 were removed

tumor size, tumor nodule location, cirrhosis, portal vein and/or microvascular tumor embolus and the histological grade of the primary nodule significantly differed between the IM and MO patients, and thus, these features were considered to be important for determining the clonal origin of multinodular HCC. Positive HBeAg, a cumulative diameter of all nodules ≤7 cm, nodules located in different lobes, cirrhosis, no portal vein or microvascular tumor embolus and/or well/moderate differentiation of the primary nodule contributed to a high rate of MO (Table 1).

The multivariate analysis using a stepwise logistic regression model further demonstrated that HBeAg was an important, but not significant, factor, but cirrhosis and portal vein and/or microvascular tumor thrombus were the independent differentiating factors between the IM and MO patients (Table 2).

Tumor-free Survival and Overall Survival between MO and IM

All patients were followed until July 2012, excluding 5 patients (2 MO patients and 3 IM patients) who received liver transplantation as the initial treatment. The median follow-up time was 13 months (range, 2 to 76 months). In the MO group, 17 patients were found to be recurrent, and 16 died. In the IM group, 18 were found to be recurrent and 18 died. The tumor-free survival time of the MO subjects was significantly longer than that of the IM subjects (25.7±4.8 months vs. 8.9±3.1 months, p=0.017) (Figure 3). Similarly, the overall survival time of the MO subjects was longer than that of the IM subjects (31.6±5.3 months vs. 15.4±3.4 months, p=0.024) (Figure 4).

The univariate analysis found that the following factors were predictive of tumor-free survival time in the IM and MO patients: hepatitis B surface antigen (HBsAg) (p=0.036), size of the primary tumor nodule (p=0.028), cumulative tumor size (p=0.008), portal vein and/or microvascular tumor thrombus (p=0.002), tumor location (p=0.007), cirrhosis (p=0.011), original type (p=0.017) and Child-Pugh grade (p<0.001). The following factors were predictive of overall survival time in the IM and MO patients: HBsAg (p=0.056), tumor size of the primary nodule (p=0.019), cumulative tumor size (p=0.003), portal vein and/or microvascular tumor thrombus (p=0.001), tumor location (p=0.063), cirrhosis (p=0.019), original type (p=0.024) and Child-Pugh grade (p<0.001).

The multivariate analysis further demonstrated that cirrhosis (p=0.055) was a poor predictive factor and that the original type (p=0.035) and Child-Pugh grade (p<0.001) were independent predictors of the tumor-free survival time in the multinodular HCC patients. Cirrhosis (p=0.011), original type (p=0.034) and Child-Pugh grade (p<0.001) were independent predictors of the overall survival time in the multinodular HCC patients (Table 3).

Discussion

Hepatocarcinogenesis is always associated with chronic liver disease, such as hepatitis B and/or hepatitis C viral infection (Chuang et al., 1992; Umemura et al., 2007; Dogan et al., 2012). Chronic inflammation increases oxidative stress and the levels of reactive oxygen species (ROS), the latter of which is believed to be one of the most important factors leading to DNA damage (Lestienne, 1992; Hibi et al., 1997; Amuthan et al., 2001). Because mtDNA lacks histones, which protect against ROS-induced DNA injury, and there is no effective DNA repair system, damage to mtDNA is more frequent and serious than damage to nuclear DNA (Croteau et al., 1997). Chronic viral inflammation is associated with repeated hepatocyte necrosis, followed by regeneration. Such an accelerated cell cycle may be associated with the accumulation of genetic errors in the liver, including mutations in the mtDNA. Recently, mtDNA mutations have been detected in human colorectal, ovarian and thyroid cancers, as well as in HCC (Carew et al., 2002; Yu, 2012). Most mutations in cancers occur in the D-loop region of mtDNA. Mutations in the mtDNA D-Loop region are a useful marker for determining the cell clonal origin for multiple HCC (Nomoto et al., 2002).

In our study, 20 patients were characterized as MO patients. The incidence rate was 47.6% (20/42), which is similar to a previous report (50%) (Utsunomiya, 2005). Of these patients, 16 had 2 cell clonality origins, and the remaining 4 patients had 3. Generally speaking, when many lesions are present in the liver, the cancer is believed to be IM (Ikeda et al., 2003). However, in our study, we found that one patient had 7 tumor nodules and yet there were 3 different sources of cell clonality origin.

In theory, tumor cells originating from the same tumor stem cell will have the same genetic variations (Calabrese et al., 2004), e.g., the same mtDNA mutations. Therefore, for tissues from the same HCC sample, the sequences of the mtDNA D-Loop region should be the same. For 16 single nodular HCC cases in the control group, 2 samples of non-consecutive tumor tissues from the same patient shared identical mtDNA D-Loop variations. Similarly, the mtDNA D-Loop variations were the same between the tumor tissue and the portal vein tumor embolus within each patient, implying that these tissues were derived from the same primary tumor nodule. In this case, the assay detecting the mtDNA D-Loop sequence was useful and feasible for differentiating MO from IM.

Shimada et al. (2001) reported that hepatitis C infection, a platelet count $<10\times10^4/\mu l$, tumor size and histological grade were independent factors for differentiating MO from IM. The report also further suggested that MO HCC, esophageal varices and liver function were independently correlated with the overall survival time of HCC patients. In another study, Nakano et al. (1994) reported that the tumor capsule was rarely observed in IM patients in contrast to MO patients and that tumor cells were more likely to invade the portal vein and the tumor capsule. In our study, HBeAg, cumulative tumor size, tumor nodule location, cirrhosis, portal vein and/or microvascular tumor embolus and the histological grade of the primary nodule significantly differed between the IM and MO patients, and thus, these were considered to be important in determining the clonal origin of multinodular HCC. The multivariate analysis further demonstrated that cirrhosis and portal vein and/or microvascular tumor thrombus were independent factors differentiating between the IM and MO patients.

HCCs with a small tumor size and well-differentiated histopathology are not believed to be metastatic, and these types of multinodular HCCs were much easier to identify as MO. Conversely, large and/or multinodular HCCs always presented a high recurrence rate, and these types of multinodular HCCs were much easier to identify as IM (Ikeda et al., 2003; Ariizumi et al., 2004). Cirrhosis was found to be the most important factor for hepatocarcinogenesis, particularly in patients with a viral hepatitis infection, and it is also believed to be an adverse risk factor (Zhou et al., 2012). As soon as the cancer appears, the cirrhotic liver enters a "highly carcinogenic state". In this "carcinogenic soil", many tumors may develop, predisposing these patients to MO (Ikeda et al., 2003).

Many studies have reported that HCV infection is an independent factor for MO HCC and that the MO occurrence rate is higher in the presence of HCV than HBV (Arii et al., 2000; Poon et al., 2000). Maeda et al. (2000) reported a higher rate of new poorly differentiated neoplasms when HBV-associated HCC recurred. However, Ikeda et al. (2003) suggested that the recurrence type of HBV-associated HCC was IM in the early period but MO in the long term. In the present study, no patient was infected with HCV, suggesting that HCV infection was not prevalent in Guangxi (a province of China). Remarkably, in this study, the rate of HBeAg positivity significantly differed between the MO and IM groups. HBeAg is a soluble protein that is closely related to DNAP and HBV DNA, and it is an important indicator of the active replication and infectious ability of HBV. Persistent positive HBeAg implies that the liver cells are seriously damaged, which has been shown to be an independent predictor of HCC recurrence and poor prognosis (Sun et al., 2007). Therefore, we speculate that an active HBV infection is an important contributor to MO, which differs from the conclusions of other studies (Arii et al., 2000; Poon et al., 2000; Ikeda et al., 2003).

The presence of tumor nodules located in different halves of the liver (right or left liver or both) appeared to significantly differ between the MO and IM groups ($p=0.040$). However, when we combined HCC patients whose tumor nodules were located in different halves of the liver (right or left liver) and compared them with the patients whose tumor nodules were located in either the right or the left liver, there was no significant difference in the prevalence of MO or IM patients ($p=0.123$). Although nodule location may not be a significant factor, this result may also have been due to the small number of included patients. Further studies should examine this relationship. A poorly differentiated primary nodule always suggests that the HCC is more invasive and metastatic; thus, multinodular HCCs may represent the IM type (Ariizumi et al., 2004). In this study, the incidence of poorly differentiated HCC was 40.9% in the IM group, while it

was 10.0% in the MO group (p=0.027). The difference remained significant even when we combined patients with well and moderately differentiated primary nodules and compared them to patients with poorly differentiated primary nodules (p=0.023). This result indicates that poor differentiation of the primary nodule contributed to the high rate of IM.

For recurrent HCCs, MO patients have been shown to have a better outcome than IM patients (Yasui et al., 1997; Huang et al., 2012). Additionally, a recurrence-free time of more than 18 months after the initial hepatic resection has been suggested as a cutoff point to differentiate MO from IM in clinical practice (Huang et al., 2012). However, in another study, a time of 2 years was suggested (Portolani et al., 2006). Similarly, for the multinodular HCCs, the survival rate of MO patients was better than the IM patients (Wang et al., 2009). In the present study, the tumor-free survival time of the MO subjects was significantly longer than that of the IM subjects (25.7±4.8 months vs. 8.9±3.1 months, p=0.017). Similarly, the overall survival time of the MO subjects was longer than that of the IM subjects (31.6±5.3 months vs. 15.4±3.4 months, p=0.024). The multivariate analysis revealed that the characteristic of the original type (IM or MO) was an independent predictor of either tumor-free survival (p=0.035) or overall survival (P=0.034). Therefore, if the liver function reserves are acceptable, it is reasonable to use locoregional therapy, such as surgical removal or radiofrequency ablation, which is more effective than transcatheter arterial chemoembolization or systemic chemotherapy, even in cases with multinodular HCCs of multicentric origin.

One drawback of the present study is that it included only 42 cases in a single center, which is not large enough to extend the conclusions to other patients. Further study should be performed with a larger number of patients at multiple centers. For this study, the determination of the original type was performed after the hepatic resection. However, in clinical practice, it is important to identify the original type of multinodular HCC preoperatively to determine the appropriate therapeutic strategy, but this is a considerable challenge. Detection of the mutations in the mtDNA D-Loop in the plasma may be useful (Nomoto et al., 2002), but further study is needed.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (NO.30960021; No.81160262/H1602). The funders had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

References

Amuthan G, Biswas G, Zhang SY, et al (2001). Mitochondria-to-nucleus stress signaling induces phenotypic changes, tumor progression and cell invasion. *EMBO J*, **20**, 1910-20.

Arii S, Yamaoka Y, Futagawa S, et al (2000). Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan. *Hepatology*, **32**, 1224-9.

Ariizumi S, Takasaki K, Yamamoto M, et al (2004). Histopathologic differentiation of the main nodule determines outcome after hepatic resection for synchronous multicentric hepatocellular carcinomas. *Hepatogastroenterology*, **51**, 500-4.

Calabrese P, Tavaré S, Shibata D (2004). Pretumor progression: clonal evolution of human stem cell populations. *Am J Pathol*, **164**,1337-46.

Carew JS, Huang P (2002). Mitochondrial defects in cancer. *Mol Cancer*, **1**, 9.

Chen YJ, Yeh SH, Chen JT, et al (2000). Chromosomal changes and clonality relationship between primary and recurrent hepatocellular carcinoma. *Gastroenterology*, **119**,431-40.

Chuang WL, Chang WY, Lu SN, et al (1992). The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area. A case-control study. *Cancer*, **69**, 2052-4.

Croteau DL, Bohr VA (1997). Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells. *J Biol Chem*, **272**, 25409-12.

Dogan E, Yalcin S, Koca D, Olmez A (2012). Clinicopathological characteristics of hepatocellular carcinoma in Turkey. *Asian Pac J Cancer Prev*, **13**, 2985-90.

Hibi K, Mitomi H, Koizumi W, et al (1997). Enhanced cellular proliferation and p53 accumulation in gastric mucosa chronically infected with Helicobacter pylori. *Am J Clin Pathol*, **108**, 26-34.

Huang ZY, Liang BY, Xiong M, et al (2012). Long-term outcomes of repeat hepatic resection in patients with recurrent hepatocellular carcinoma and analysis of recurrent types and their prognosis: a single-center experience in china. *Ann Surg Oncol*, **19**, 2515-25.

Ikeda K, Arase Y, Kobayashi M, et al (2003). Significance of multicentric cancer recurrence after potentially curative ablation of hepatocellular carcinoma: a long term cohort study of 892 patients with viral cirrhosis. *J Gastroenterol*, **38**, 865-76.

Lestienne P (1992). Mitochondrial DNA mutations in human diseases: a review. *Biochimie*, **74**, 123-30.

Li Q, Wang J, Juzi JT, et al (2008). Clonality analysis for multicentric origin and intrahepatic metastasis in recurrent and primary hepatocellular carcinoma. *J Gastrointest Surg*, **12**, 1540-7.

Liver Cancer Study Group of Japan (1997). Classification of primary liver cancer. First English Ed, Kanehara Shuppan,Tokyo.

Maeda T, Takenaka K, Taguchi K, et al (2000). Clinicopathological characteristics of surgically resected minute hepatocellular carcinomas. *Hepatogastroenterology*, **47**, 498-503.

Nakano S, Haratake J, Okamoto K, Takeda S (1994). Investigation of resected multinodular hepatocellular carcinoma: assessment of unicentric or multicentric genesis from histological and prognostic viewpoint. *Am J Gastroenterol*, **89**, 189-93.

Nomoto S, Kinoshita T, Kato K, et al (2007). Hypermethylation of multiple genes as clonal markers in multicentric hepatocellular carcinoma. *Br J Cancer*, **97**, 1260-5.

Nomoto S, Yamashita K, Koshikawa K, Nakao A, Sidransky D (2002). Mitochondrial D-loop mutations as clonal markers in multicentric hepatocellular carcinoma and plasma. *Clin Cancer Res*, **8**, 481-7.

Oda T, Tsuda H, Scarpa A, Sakamoto M, Hirohashi S (1992). Mutation pattern of the p53 gene as a diagnostic marker for multiple hepatocellular carcinoma. *Cancer Res*, **52**, 3674-8.

Okamoto M, Utsunomiya T, Wakiyama S, et al (2006). Specific

gene-expression profiles of noncancerous liver tissue predict the risk for multicentric occurrence of hepatocellular carcinoma in hepatitis C virus-positive patients. *Ann Surg Oncol*, **13**, 947-54.

Parkin DM, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J Clin*, **55**, 74-108.

Perry JF, Charlton B, Koorey DJ, et al (2007). Outcome of patients with hepatocellular carcinoma referred to a tertiary centre with availability of multiple treatment options including cadaveric liver transplantation. *Liver Int*, **27**, 1240-8.

Poon RTP, Fan ST, Wong J (2000). Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma. *Ann Surg*, **232**, 10-2.

Portolani N, Coniglio A, Ghidoni S, et al (2006). Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. *Ann Surg*, **243**, 229-35.

Shimada M, Hamatsu T, Yamashita Y, et al (2001). Characteristics of multicentric hepatocellular carcinomas: comparison with intrahepatic metastasis. *World J Surg*, **25**, 991-5.

Sun HC, Zhang W, Qin LX, et al (2007). Positive serum hepatitis B e antigen is associated with higher risk of early recurrence and poorer survival in patients after curative resection of hepatitis B-related hepatocellular carcinoma. *J Hepatol*, **47**, 684-90.

Umemura T, Kiyosawa K (2007). Epidemiology of hepatocellular carcinoma in Japan. *Hepatol Res*, **37** Suppl 2, S95-S100.

Utsunomiya I (2005). Pathological study on multicentric occurrence of hepatocellular carcinoma. *Kurume Med J*, **52**, 133-8.

Wang J, Li Q, Sun Y, et al (2009). Clinicopathologic features between multicentric occurence and intrahepatic metastasis of multiple hepatocellular carcinomas related to HBV. *Surg Oncol*, **18**, 25-30.

Yamamoto T, Kajino K, Kudo M, et al (1999). Determination of the clonal origin of multiple human hepatocellular carcinomas by cloning and polymerase chain reaction of the integrated hepatitis B virus DNA. *Hepatology*, **29**, 1446-52.

Yasui M, Harada A, Nonami T, et al (1997). Potentially multicentric hepatocellular carcinoma: clinicopathologic characteristics and postoperative prognosis. *World J Surg*, **21**, 860-5.

Yu M (2012). Somatic mitochondrial DNA mutations in human cancers. *Adv Clin Chem*, **57**, 99-138.

Zhou L, Liu C, Meng FD, et al (2012). Long-term prognosis in hepatocellular carcinoma patients after hepatectomy. *Asian Pac J Cancer Prev*, **13**, 483-6.