ANTICANCER RESEARCH *39*: 4315-4324 (2019)
doi:10.21873/anticanres.13597

Microsatellite Analysis of Recurrent Lesions Confirms Merit of Anatomical Liver Resection for Hepatocellular Carcinoma

YUHKI SAKURAOKA[1], KEIICHI KUBOTA[1], JOHJI IMURA[2], HIDETSUGU YAMAGISHI[3], TAKU AOKI[1], TAKATSUGU MATSUMOTO[1], TAKAO ARAKAWA[1], TAKASHI SUZUKI[1], GENKI TANAKA[1], TAKAYUKI SHIMIZU[1], KAZUMA TAGO[1], KYUNG HWA PARK[1], TAKAYUKI SHIRAKI[1], SHOZO MORI[1], YUKIHIRO ISO[1] and MASATO KATO[1]

[1]*Second Department of Surgery, Dokkyo Medical University, Tochigi, Japan;*
[2]*Department of Diagnostic Pathology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan;*
[3]*Department of Diagnostic Pathology, Dokkyo Medical University, Tochigi, Japan*

Abstract. *Background/Aim: This study aimed to obtain accurate differential diagnosis (DDx) of multicentric carcinogenesis (MC) and intrahepatic metastasis (IM) in recurrent lesions of hepatocellular carcinoma. Materials and Methods: A total of 79 patients who underwent re-hepatectomy (2000-2013) were examined. PCR was used to analyze 13 chromosomal microsatellite loci by PCR. On the basis of this genetic analysis, the recurrent lesions were diagnosed as IM, MC or not determined (ND). Subsequently, DDx was compared with types of resection and outcome. Results: The recurrent lesions were diagnosed as IM in 33 patients, MC in 44, and ND in 2. The anatomical resection group included 14 IM lesions (28%) and 36 MC lesions (72%), while the non-anatomical resection group included 19 IM lesions (70%) and 8 MC lesions (30%) ($p<0.001$). Conclusion: Anatomical resection at initial hepatectomy may reduce the likelihood of IM recurrence, leading to a better outcome for patients with HCC.*

Recurrence of hepatocellular carcinoma (HCC) after hepatic resection may be in the form of either multicentric carcinogenesis (MC) or intrahepatic metastasis (IM) (1), and clinical differentiation between them is difficult. The General Rules for the Clinical and Pathological Study of Primary Liver Cancer (2, 3) consider that daughter lesions should be diagnosed as IM if they have components of portal vein tumour thrombi, or if tumours are growing in apparent contiguity with these thrombi. This means that IM lesions are distributed as multiple small satellite nodules surrounding a larger primary tumour. On the other hand, when the histopathological diagnosis is primary or recurrent well-differentiated HCC, the tumour is usually considered to be MC, as metastasis from well-differentiated HCC is infrequent (3). Currently, there is no gold-standard diagnostic criterion for determining whether such a recurrent lesion has originated from IM or MC. Moreover, >40% of postoperatively recurrent HCCs exhibit intranodular vascular changes, making the findings of computed tomography inconsistent between primary and recurrent HCC (4). For this reason, the ability of diagnostic imaging to differentiate between IM and MC is limited. In this context, genetic diagnosis using microsatellite analysis may be more useful than histopathological diagnosis (5), as this allows evaluation of tumour clonality (6). In general, decisions pertaining to hepatic resection are based on liver function and remnant liver volume. Several studies have demonstrated that anatomical resection provides longer relapse-free and overall survival periods (7-9), although the reason for this remains unclear and is contentious. In the present study we, therefore, examined the accuracy of differential diagnosis (DDx) using genetic analysis of microsatellites in recurrent HCC, and explored the reasons why anatomical resection has a positive impact on prognostic outcome.

Correspondence to: Yuhki Sakuraoka, Second Department of Surgery, Dokkyo Medical University, 880 Kitakobayashi, Mibu, Tochigi 321-0293, Japan. Tel: +81 282861111, Fax: +81 282866317, e-mail: sakuraoka812@gmail.com

Key Words: Intrahepatic metastasis, multicentric carcinogenesis, microsatellite, hepatocellular carcinoma, recurrence.

Patients and Methods

Patients. The retrospective design of this study (0054629) was reviewed and approved by the institutional review board. Between April 2000 and October 2013, 537 patients with primary HCC underwent anatomical or non-anatomical resection for the first time at our department. After excluding cases involving multiple recurrent lesions and relapse-free patients, a total of 79 patients with

one recurrent lesion who subsequently underwent repeat hepatectomy on the basis of our departmental criteria (described below) were included in the study (Figure 1).

Liver resection. The indications for hepatic resection and the type of operative procedure used were based on the criteria of Makuuchi (10). Liver function was evaluated using the indocyanine green retention rate (0.5 mg/kg) at 15 min (ICGR15), and a value of <10% was considered normal (11). In addition, the presence or absence of ascites and the serum total bilirubin level were considered important factors for selection of the hepatectomy procedure. The resection procedure, either anatomical or non-anatomical, was selected based on the patient's liver function and remnant liver volume. In principle, non-anatomical resection is employed for re-hepatectomy.

Tissue samples. Paraffinized 10-μm-thick sections of the primary and recurrent HCC specimens and the corresponding non-tumorous liver tissues were used for PCR analysis. The tissues had been previously fixed using buffered formalin, embedded in paraffin using standard methods, and confirmed to be HCC based on histopathological examination. The sections were deparaffinised and digested using proteinase K, and the genomic DNA of each sample was isolated using a QIAamp tissue kit (Qiagen Inc. Venlo, Netherlands).

Microsatellite analysis. We examined 13 chromosomal microsatellite loci (D1S233, D1S407, D3S1539, D8S277, D8S261, D9S156, D9S254, D10S520, D13S284, D16S515, D4S1554, D17S513, and TP53) in 237 specimens (non-cancerous liver tissue, carcinoma tissue from the first hepatectomy, and carcinoma tissue from the re-hepatectomy) from 79 patients to detect loss of heterozygosity (LOH) on the chromosome arms using relatively frequent HCC loci that had been described in previous reports (12-23). The PCR primers were synthesised based on the genome sequences in a genome database. PCR was performed using a HotStarTaq Plus Master Mix Kit with >10 ng of the extracted genomic DNA. The reaction was performed in a thermocycler, with an initial denaturation of 5 min at 94˚C, followed by 30 cycles of 94˚C for 1 min, 63˚C for 1 min, and 72˚C for 1 min, and a final elongation of 10 min at 72˚C. The PCR products were analysed using an Agilent 2100 bioanalyser.

The existence of LOH or microsatellite instability (MSI) was determined by comparing the primary or recurrent tumour tissues with the corresponding normal liver tissues. LOH is a gross chromosomal event that results in loss of the entire gene and the surrounding chromosomal region (24). MSI is a condition of genetic hypermutability that results from impaired DNA mismatch repair. The presence of MSI is phenotypic evidence that mismatch repair is not functioning normally (25). When two or more amplified bands per locus were detected in normal liver tissues, the case was defined as informative for the analysis. For every informative marker, LOH was defined as one of the two bands being absent when tumour tissue was compared to normal tissue. MSI was defined as a band shift in one or two alleles or the presence of novel bands in the two alleles in the tumour samples (Figure 2). When the band pattern was the same between the primary lesion and recurrent lesion, the lesion was diagnosed as retention (R) because these lesions exhibited retention of heterozygosity and retained the same genetic characteristics in the locus.

Each specimen was classified as retention R, LOH, MSI, or not determined (ND). When a change from MSI to R and/or LOH to R and/or LOH to LOH at another site was detected, the lesion was diagnosed as MC. When there were no changes (including R to LOH or MSI), the lesion was diagnosed as IM. Ambiguous cases were diagnosed as ND (Table I). Based on the diagnostic findings regarding IM and MC, computed tomography findings, pathological findings, overall survival, recurrence-free survival, and hepatectomy procedures (anatomical or non-anatomical) were compared between the two groups.

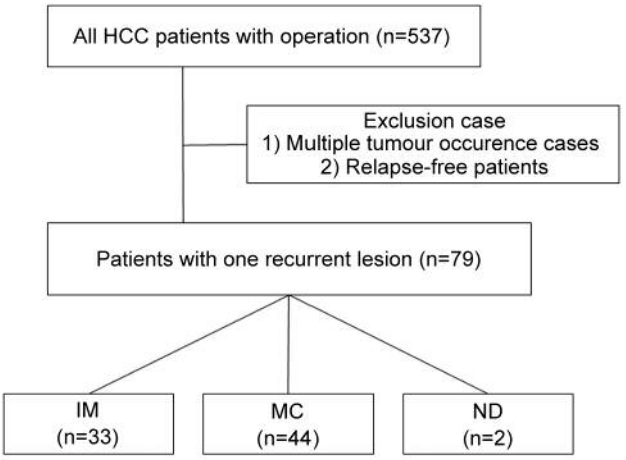

Figure 1. *Flow diagram of study. A total of 537 patients with primary HCC underwent anatomical or non-anatomical resection for the first time at our department. After excluding patients with multiple recurrent lesions and patients who were relapse-free, a total of 79 patients with one recurrent lesion who subsequently underwent repeat hepatectomy on the basis of our departmental criteria were included in the study.*

Ethical approval and consent to participate. All procedures performed in this study were in accordance with the ethical standards of the ethics committee of Dokkyo Medical University (approved number 0054629) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statistical analysis. All data were analysed using SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA). Continuous variables are reported as the median and range, and were compared using the Mann-Whitney *U*-test. Categorical variables are reported as a number and percentage, and were compared using Fisher's exact test. Multivariate logistic regression analyses were used to identify risk factors for pathological portal vein invasion. The disease-free and overall survival rates were calculated using the Kaplan–Meier method and compared using the log-rank test. Univariate and multivariate analyses were performed to identify risk factors for HCC recurrence with pathologic portal vein invasion using the Cox regression model. Differences at $p<0.05$ were considered statistically significant.

Results

Patient characteristics (Table II). The 79 patients included 60 men and 19 women, with a median age of 67 years; 69 of 79 patients suffered from viral hepatitis B or C. Fourteen

Table I. *Diagnostic scheme. When LOH to R and/or LOH to LOH was detected, the lesion was diagnosed as MC. When there were no changes including R to LOH or MSI, the lesion was diagnosed as IM. When it was difficult to assign the lesion to either group, it was diagnosed as ND. When R to LOH and/or R to MSI was detected, the lesion was diagnosed as ND. Similarly, when MSI to MSI (MSI*) was detected, the lesion was diagnosed as ND.*

Primary lesion	Recurrent lesion	Diagnosis
LOH	R	MC
LOH	LOH*	
MSI	R	
LOH	LOH	IM
R	R	
MSI	MSI	
R	LOH	ND
R	MSI	
MSI	MSI*	

Table II. *Patient characteristics at the first hepatectomy.*

Characteristics	Total patients (n=79)
Age: yr	41-84(Median:66.5)
Gender: male/female	60/19
Viral infection	
HBV(+)HCV(–)	17
HBV(–)HCV(+)	51
HBV(+)HCV(+)	1
HBV(–)HCV(–)	10
Tumor grade	
Well differentiated	17
Moderately differentiated	56
Poorly differentiated	6
Liver histology	
Normal (f0)	4
Chronic hepatitis (f1, f2)	23
Cirrhosis (f3, f4)	52
ICG15	
<10%	14
10-20%	39
>20%	26
Stage	
I	15
II	38
III	21
IV	5
Operation	
Anatomical resection	50
Non-anatomical resection	29

HBV: Hepatitis B virus; HCV: hepatitis C virus; F: fibrosis of the liver.

patients had normal liver function, and 65 patients had impaired liver function (ICGR15 >10%) (26, 27). There were no cases of postoperative liver failure or death after repeated

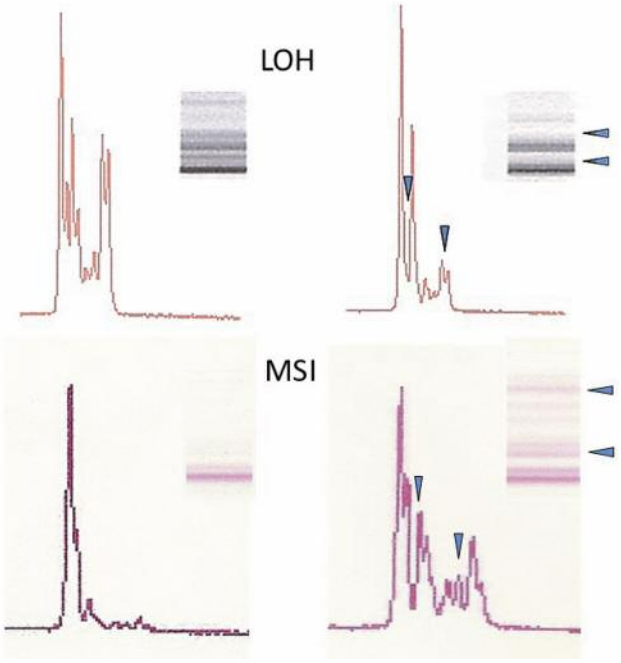

Figure 2. *Typical electophoretic patterns of LOH at D1S233 and MSI at D16S515 in case 26. LOH was defined as one of the two bands being absent (arrowheads indicate the disappearance of the two bands) in tumour tissues compared to normal tissues. MSI was defined as a band shift in one or two alleles or the presence of novel bands in the two alleles (arrowheads show the appearance of the two novel bands) in tumour samples.*

hepatectomy. For initial hepatectomy, 50 patients underwent anatomical resection (hepatic lobectomy, segmentectomy, and subsegmentectomy), and 29 underwent non-anatomical resection (partial resection). Based on the AJCC/UICC disease staging system (28), 15 patients were stage I, 38 were stage II, 21 were stage III, and 5 were stage IV. In terms of tumour differentiation, 17 patients had well differentiated tumours, 56 had moderately differentiated tumours, and 6 had poorly differentiated tumours.

Comparison between IM and MC. Among the 79 patients, 44 were diagnosed as having MC lesions and 33 as having IM lesions, although 2 cases remained ND after analysis (Figure 3).

i) Clinicopathological data. The anatomical resection group included 14 IM lesions (28%) and 36 MC lesions (72%), while the non-anatomical resection group included 19 IM lesions (70%) and 8 MC lesions (30%) (p=0.0003). The incidence of IM and MC lesions was associated with anatomical or non-anatomical resection. However, the incidence of IM and MC was not influenced by any pathological features after primary resection (tumour-related factors, normal liver factors, tumour grade, growth pattern,

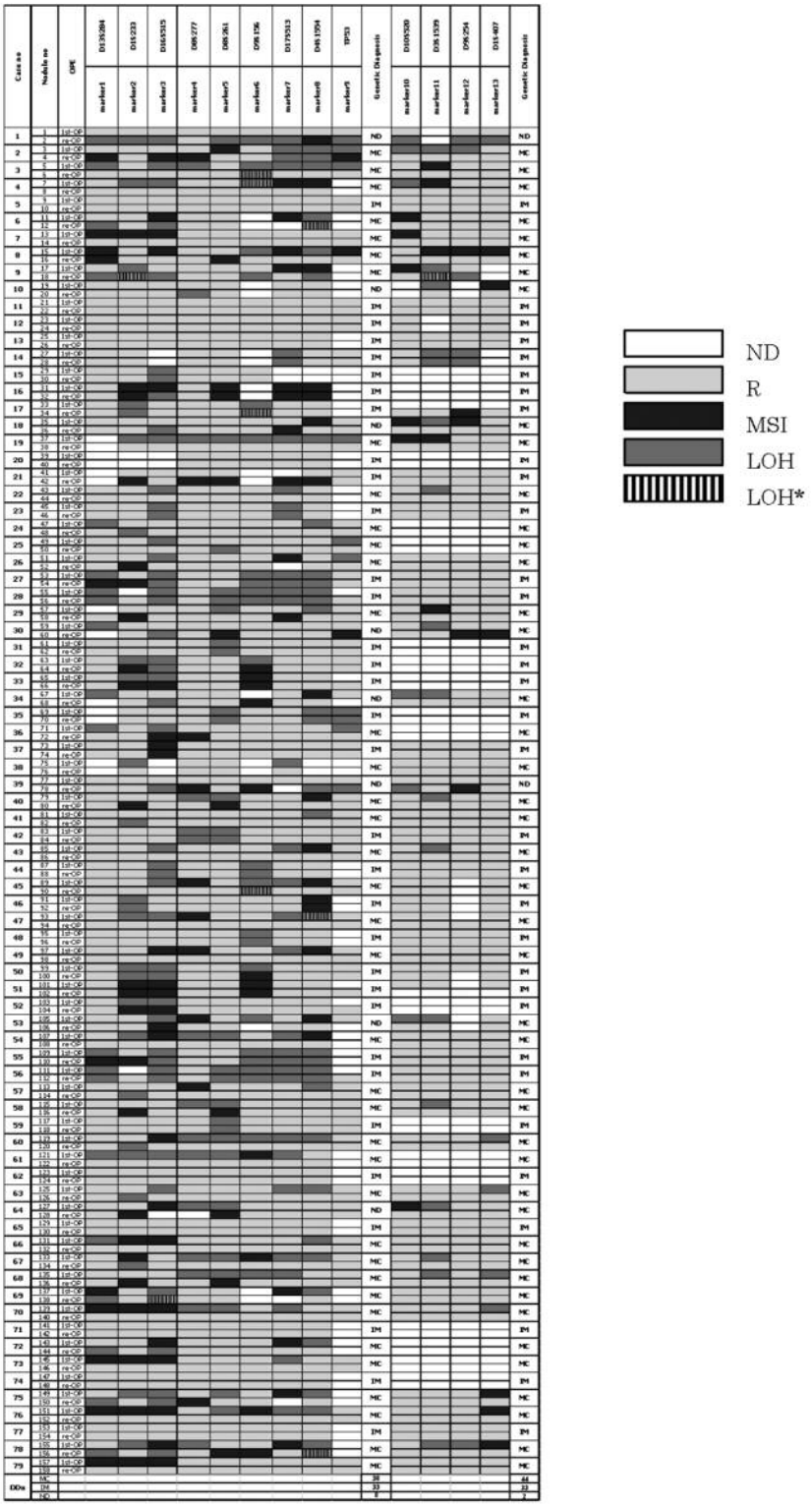

Figure 3. *Results of analysis of 13 microsatellites. Thirteen chromosomal microsatellite loci (D1S233, D1S407, D3S1539, D8S277, D8S261, D9S156, D9S254, D10S520, D13S284, D16S515, D4S1554, D17S513 and TP53) were analyzed using the PCR-based microsatellite polymorphic method in 79 patients (237 lesions: non-cancer liver tissue, carcinoma at first hepatectomy and carcinoma at re-hepatectomy, respectively), and were assigned to retention (R), loss of heterozygosity (LOH), LOH at the other site (LOH*), microsatellite instability (MSI) or not detected (ND). They were then diagnosed by microsatellite analysis.*

capsule formation, capsule infiltration, septum formation, and portal vein infiltration) (Table III).

ii) CT findings (Table IV). The changes in computed tomography findings are summarised in Table IV; none of the patterns of change were associated with MC or IM, and these lesions were difficult to differentiate based on imaging findings alone. Specifically, 32 of 33 IM cases and 42 of 44 MC cases showed the same low-high-low pattern as the primary lesion. Other than these cases, there was a small number of examples of other patterns.

iii) Pathological findings (Table V). The changes in pathological differentiation are summarised in Table V; it was difficult to differentiate between IM and MC lesions based on changes in pathological differentiation, even though several patterns of pathological changes were evident, as shown in Table V. Specifically, 21 IM cases showed moderately differentiated adenocarcinoma in the primary lesions and the same differentiated status in the recurrent lesions. In 16 IM cases, the primary lesions showed moderately differentiated adenocarcinoma, and the recurrent lesions revealed well differentiated adenocarcinoma. A change from well differentiated to moderately differentiated adenocarcinoma was found in 8 patients. On the other hand, in 21 MC cases, the same moderately differentiated adenocarcinoma status was evident in both the primary and secondary lesions.

Outcome. The median overall survival and relapse-free survival periods were 116 months [95% confidence interval (CI)=107-125] and 20 months (95%CI=12-29), respectively (Figure 4a and b). The 1-, 3-, and 5-year overall survival rates and relapse-free survival rates after the first hepatic resection were 98.7%, 90.9% and 77.9%, and 74.0%, 41.6%, and 16.9%, respectively (Figure 4a and b). The median overall survival periods for patients with IM and MC were 114.3 months and 151.4 months, respectively (p=0.0454), and the median relapse-free survival times were 13.5 and 28.2 months (p<0.0001), respectively (Figure 4c and d). The correlations between the types of recurrence and survival of the 77 patients (33 IM cases and 44 MC cases) are shown in Figure 4c and d. Cox regression analysis revealed that patients with MC exhibited a higher overall survival rate than those with IM (hazard ratio=1.830, 95%CI=1.013-3.309). Patients with MC also exhibited a higher relapse-free survival rate than those with IM (hazard ratio=8.794, 95%CI=4.593-16.84).

Table III. *Clinicopathological correlations between IM and MC*.

Primary lesion parameter	IM (n=33)	MC (n=44)	p-Value	Odds ratio	95%Cl
Resection					
Anatomical	14	36	0.0003	6.11	2.18-17.13
Non-anatomical	19	8			
Non-tumorous liver					
Normal	3	4			
Chronic hepatitis	9	12	0.53	-	-
Cirrhosis	21	28			
Tumor grade					
Well differentiated	7	10			
Moderately differentiated	23	31	0.93	-	-
Poorly differentiated	3	3			
Growth Pattern					
Eg	31	43	0.40	0.36	0.27-1.46
Ig	2	1			
Formation of capsule					
Fc–	11	18	0.50	0.72	0.28-1.85
Fc+	22	26			
Infiltration of capsule					
Fc-Inf–	21	30	0.68	0.82	0.32-2.11
Fc-Inf+	12	14			
Septum formation					
Sf–	14	21	0.64	0.81	0.33-2.00
Sf+	19	23			
Portal vein invasion					
Vp–	28	36	0.73	1.24	0.39-4.22
Vp+	5	8			

Eg: Expansive growth; Ig: infiltrative growth; Fc: capsule formation; Fc-Inf: cancerous infiltration of the capsule; Sf: formation of fibrous septum within the tumor; Vp: portal vein invasion.

Discussion

This study investigated the effectiveness of DNA microsatellite analysis for DDx of recurrent HCC in relation to clinical features. The results suggested that microsatellite analysis might be the only means to differentiate between MC and IM accurately, and that anatomical resection at initial hepatectomy may reduce the likelihood of IM recurrence, leading to a better outcome.

Microsatellite analysis is used for genetic fingerprinting of individuals, and previous reports have suggested that it is useful for identifying specific oncological features. In relation to HCC, Morimoto *et al*. first used microsatellite analysis to distinguish between IM and MC in 2003 and demonstrated that genetic diagnosis may be the most accurate approach for DDx between IM and MC. In 2008, this technique was proved to be accurate for distinguishing between MC and IM on the basis of clonality (6). Some more recent studies have revealed that a high frequency of MSI in any type of cancer is strongly correlated with the effect of PD-1 inhibitors (29, 30).

Accordingly, in the present study, we investigated the clinical features of IM and MC and the accuracy of DDx using microsatellite analysis. In clinical settings, however, microsatellite analysis may not be suitable as a standard method for differentiating between IM and MC in view of its labor intensiveness and high cost. Therefore, 13 microsatellite markers, previously proven to be representative markers of

Table IV. *Changes in CT imaging patterns were analysed based on density level determined by dynamic CT. Arterial, portal and venous phases are shown in sequence.*

Primary lesion	Recurrent lesion	IM (n=33)	MC (n=44)	ND (n=2)	*p*-Value
Low-High-Low	Low-High-Low	32	42	-	
Low-Iso-Low	Low-High-Low	1	-	1	n.s
Low-Iso-Low	Low-Iso-Low	-	1	1	
Low-High-Low	Low-Iso-Low	-	1	-	

Low: Low-density lesion; Iso: isodense lesion; High: high-density lesion.

Table V. *Changes in the pathological patterns.*

	IM (n=33)		MC (n=44)		ND (n=2)		*p*-Value
Pathological difference patterns	Mod→Mod	21	Mod→Mod	20	Mod→Mod	1	
	Well→Mod	1	Well→Mod	8	Well→Mod	1	
	Mod→Well	8	Mod→Well	16			n.s
	Mod→Por	1					
	Por→Mod	1					
	Well→Well	1					

Well: Well differentiated carcinoma; Mod: moderately differentiated carcinoma; Por: poorly differentiated carcinoma; n.s: not statistically significant.

LOH and MSI were carefully selected (10-21). First, 9 microsatellite markers were analyzed, but DDx was achieved in only 71 cases (88.9%). To achieve diagnosis in ND cases, we added 4 additional microsatellite markers, and this allowed us to achieve DDx in a final total of 77 cases (97.5%). Of course, it may have been possible to distinguish IM from MC in the remaining 2 ND cases if more markers had been employed. In general, the frequency of MSI and LOH in HCC is low. Because our study was based on the occurrence of MSI and LOH in microsatellite markers, we considered the total of 13 markers to be the limit for this method. Admittedly, however, innovative next-generation sequencing has now made it possible to obtain genetic information on a more individual basis, and thus more accurate distinction between IM and MC might be feasible. In fact, using next-generation sequencing, Furuta *et al.* have already reported the genetic features of IM and MC in 24 patients (31).

As mentioned above, as patients with severe liver dysfunction cannot undergo anatomical resection, distinction between IM and MC has no relevance for selecting the type of liver resection. In the present study, however, we were not only able to distinguish between IM and MC accurately, but also demonstrated a relationship between anatomical resection and the mode of recurrence: patients who had undergone anatomical resection were more likely prone to have recurrence in the form of MC and longer relapse-free survival. This appears to provide, for the first time, a plausible rationale for performing anatomical resection on the basis of genetic analysis, on the condition that patients have sufficiently good liver function. In this context, short-term and careful observation after non-anatomical resection would be desirable for earlier discovery of recurrent IM lesions.

Some previous studies have identified high rates of IM in larger tumours (>90% for those with a diameter of ≥5 cm), suggesting a close relationship between IM and portal vein invasion for such tumours (32-37). Nevertheless, the mechanisms underlying these clinical features have remained unclear, and previous studies did not address recurrence after removal of the first primary HCC. Consequently, no criteria for distinguishing between IM and MC recurrence have been established.

With regard to CT findings, most of the primary and recurrent lesions showed the same low-high-low hemodynamic pattern, which reduced the utility of CT for DDx. With regard to pathology, even though the primary lesion was moderately differentiated adenocarcinoma in 8 of 33 IM cases, the recurrent lesions were well differentiated. This finding was surprising because on the basis of oncological principles we had expected that IM from a well differentiated primary tumour would have shown a moderately or poorly differentiated histology. Although the reason for this remained unclear, it must be acknowledged that the potential for differentiation of a recurrent tumour is not determined by the whole of the primary tumour tissue. Therefore, diagnosis of differentiation on the basis of

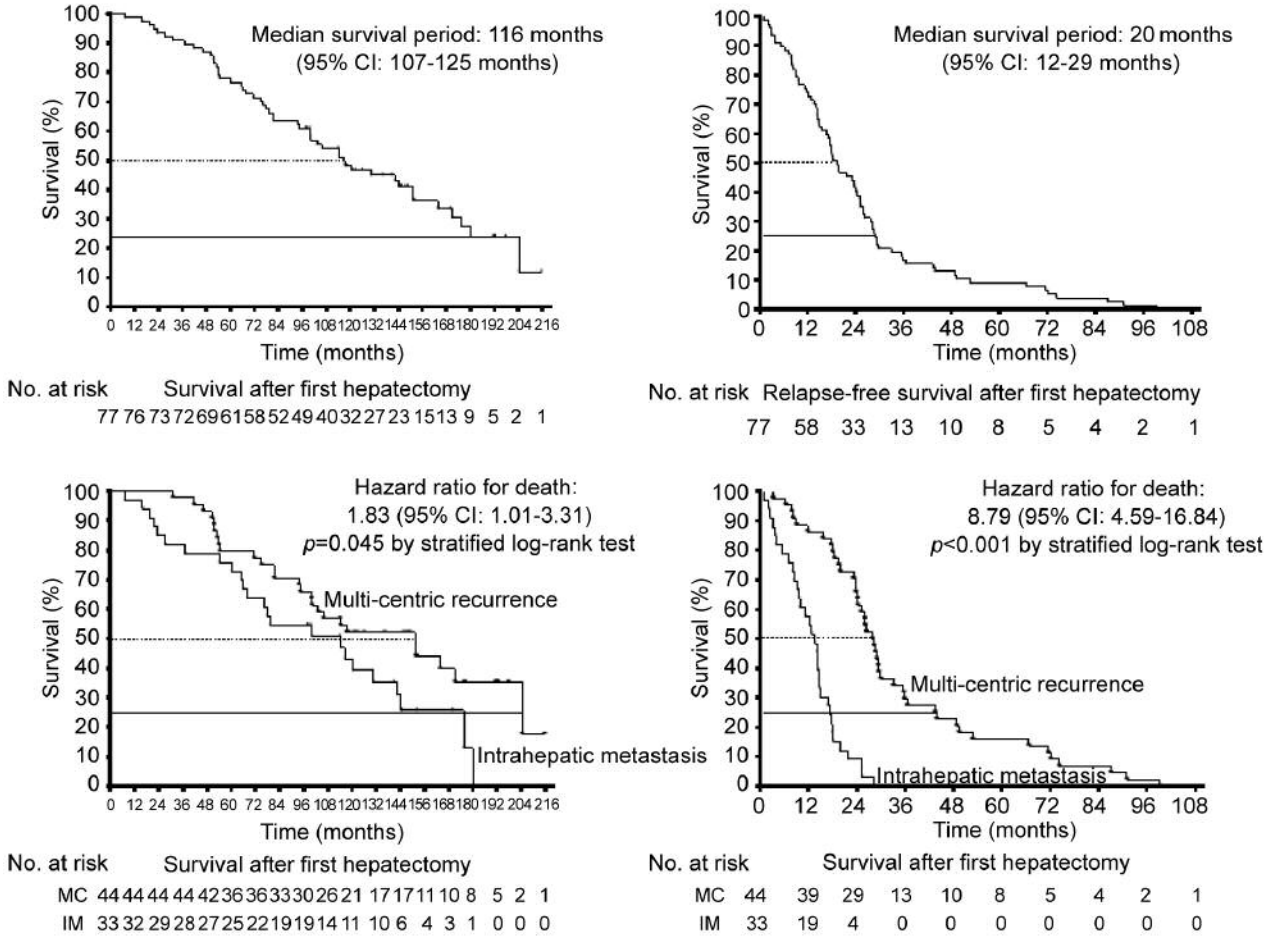

Figure 4. *Overall survival, relapse-free survival. Overall survival according to IM and MC and relapse-free survival according to IM and MC. The median overall survival and relapse-free survival rates were 116 months [95% confidence interval (CI) 107-125 months] and 20 months (95%CI=12-29 months), respectively (a and b). The 1-, 3-, and 5-year overall survival rates and relapse-free survival rates after initial hepatic resection were 98.7%, 90.9% and 77.9%, and 74.0%, 41.6%, and 16.9%, respectively (a and b). The median overall survival periods of patients with MC and IM were 151.4 and 114.3 months, respectively, the difference being significant (p=0.0454) (c). The median relapse-free survival periods for MC and IM patients were 28.2 and 13.5 months, respectively (p<0.0001) (d). Cox regression analysis also demonstrated a significant difference between the two groups. The overall survival rate of MC patients was significantly higher than that of IM patients (HR 1.830, 95%CI=1.013-3.309) and the relapse-free survival rate of MC patients was significantly higher than that of IM patients (HR 8.794, 95%CI=4.593-16.84).*

isolated parts of solid tumours may not be accurate. Another unexpected finding was that in 20 of 44 MC cases, moderately differentiated adenocarcinoma showed no change in histological differentiation. This made it difficult to differentiate between IM and MC based on histological differentiation alone, and our findings suggested that both methods are inadequate for making an accurate DDx.

With regard to the prognosis of patients with HCC recurrence, some studies have reported that the outcome of those with MC is significantly better than that of those with IM (38-41). Moreover, several meta-analyses have reported that anatomical resection (AR) is superior to non-anatomical resection (NAR) of HCC in terms of survival rate and local recurrence (42, 43). However, no reports have clearly described the relationship between cause and effect, or provided proof based on genetic data.

Subgroup analyses revealed that patients who underwent AR were more likely to suffer MC recurrence than IM recurrence, while NAR tended to be associated with a higher incidence of IM recurrence. In other words, AR appeared to reduce IM recurrence. Admittedly, the multivariate Cox regression model was not applied because our sample size was small and thus inappropriate. However, on the basis of an approach that yields an accurate DDx, this may be the first prospective study to have provided evidence to explain the prognostic superiority of AR over NAR. Additionally, our

findings indicate that MC is associated with a longer recurrence-free survival period than is the case for IM. One possible reason for this may be that the tumour-feeding vessel is normally an artery, and that the portal venous system provides drainage, which may explain why these tumours exhibit intra-portal invasion at an early stage. This invasion may also contribute to the high rate of IM. Thus, anatomical resection may extirpate potential IM lesions, reducing the risk of IM from HCC, which would help prolong both the recurrence-free and overall survival periods. In other words, suppression of IM, which is predicted to recur along the portal vein, may help extend subsequent recurrence-free survival.

On the other hand, several study limitations should also be acknowledged. First, we did not consider the surgical margin, as we deliberately excluded cases with no surgical margin. A sufficient amount of both normal liver tissue and carcinoma is required to obtain a sufficient amount of DNA for accurate genetic analysis. If there had been no surgical margins in the paraffin sections, sufficient amount of DNA could not have been obtained. In addition, because the study was focused on the relationship between outcome and type of resection based on accurate diagnosis, exclusion of cases with no surgical margins was considered reasonable and proper for the purposes of this study. Second, just one recurrent lesion was chosen for analysis for two major reasons. One is that we were unable to obtain tissue samples from cases involving multiple foci of recurrence. This is because, for oncological reasons, patients having multiple recurrent lesions are not eligible for resection. The other reason is that we considered the number of cases requiring analysis would be extremely large. Hepatectomy and re-hepatectomy are usually performed in patients with fewer than three tumours. If all of these lesions had been included, a large number of possible combinations of patterns would have been created for genetic analysis. For this reason, in the present study, we only focused on cases involving a single recurrent lesion to allow accurate comparison of the primary and recurrent lesions using microsatellite analysis.

In conclusion, microsatellite analysis of HCC can allow for accurate differentiation between MC and IM. Recurrent lesions after anatomical resection are more likely to be MC, whereas recurrence after non-anatomical resection is more likely to be IM. Although the number of patients examined was limited and further assessment of accumulated recurrent cases will be necessary, our results suggest that recurrence of HCC soon after initial hepatectomy is likely to be IM and that selection of anatomical resection as a procedure for initial hepatectomy may help reduce the likelihood of IM recurrence.

Conflicts of Interest

The Authors declare no competing interests.

Authors' Contributions

K.K. and J.I. provided the main concept of this study. H. Y. collected tissue sample. The other co-responding authors collected the data and performed statistical analysis. Y.S. wrote the first version of the manuscript. The final version of the manuscript was approved by everyone.

Acknowledgements

This study, especially for DNA sampling, was performed by well-established technician in Dokkyo Medical University and supported by all members of Second Department of Surgery.

References

1 Bi C, Wang LM, An SL, Huang J, Feng RM, Wu F, Rong WQ and Wu JX: Analysis of the survival of 123 patients with intrahepatic cholangiocarcinoma after surgical resection. Zhonghua Zhong Liu Za Zhi *38(6)*: 466-471, 2016. PMID: 27346406. DOI: 10.3760/cma.j.issn.0253-3766

2 Kudo M, Kitano M, Sakurai T and Nishida N: General rules for the clinical and pathological study of primary liver cancer. nationwide follow-up survey and clinical practice guidelines: the outstanding achievements of the liver cancer study group of Japan. Dig Dis *33(6)*: 765-770, 2015. PMID: 26488173. DOI: 10.1159/000439101

3 Kudo M, Kitano M, Sakurai T and Nishida N: General rules for the clinical and pathological study of primary liver cancer, nationwide follow-up survey and clinical practice guidelines: The outstanding achievements of the Liver Cancer Study Group of Japan. Dig Dis *33(6)*: 765-770, 2015. PMID: 26488173. DOI: 10.1159/000439101

4 Tajima T, Yoshimitsu K, Irie H, Aibe H, Shinozaki K, Nishie A, Honda H and Shimada M: Detecting postsurgical recurrent hepatocellular carcinoma with multiphasic helical computed tomography: intrahepatic metastasis or multicentric occurrence? J Comput Assist Tomogr *29(1)*: 42-50, 2005. PMID: 15665682.

5 Morimoto O, Nagano H, Sakon M, Fujiwara Y, Yamada T, Nakagawa H, Miyamoto A, Kondo M, Arai I, Yamamoto T, Ota H, Dono K, Umeshita K, Nakamori S, Sasaki Y, Ishikawa O Imaoka S and Monden M: Diagnosis of intrahepatic metastasis and multicentric carcinogenesis by microsatellite loss of heterozygosity in patients with multiple and recurrent hepatocellular carcinomas. J Hepatol *39(2)*: 215-221, 2003. PMID: 12873818.

6 Ng IO, Guan XY, Poon RT, Fan ST and Lee JM: Determination of the molecular relationship between multiple tumour nodules in hepatocellular carcinoma differentiates multicentric origin from intrahepatic metastasis. J Pathol *199(3)*: 345-353, 2003. PMID: 12579536. DOI: 10.1002/path.1287

7 Kim S, Kim S, Song I and Chun K: Comparison of survival outcomes after anatomical resection and non-anatomical resection in patients with hepatocellular carcinoma. Korean J Hepatobiliary Pancreat Surg *19(4)*: 161-166, 2015. PMID: 26693235. DOI: 10.14701/kjhbps

8 Matsumoto T, Kubota K, Aoki T, Iso Y, Kato M and Shimoda M: Clinical impact of anatomical liver resection for hepatocellular carcinoma with pathologically proven portal vein invasion. World J Surg *40(2)*: 402-411, 2016. PMID: 26306893. DOI: 10.1007/s00268-015-3231-1

9 Yamamoto T, Yagi S, Kita R, Masui H, Kinoshita H, Sakamoto Y, Okada K, Miki A, Kondo M, Hashida H, Kobayashi H, Uryuhara K, Kaihara S and Hosotani R: Comparison between anatomical subsegmentectomy and nonanatomical partial resection for hepatocellular carcinoma located within a single subsegment: a single-center retrospective analysis. Hepatogastroenterology *62(138)*: 363-367, 2015. PMID: 25916064.

10 Miyagawa S, Makuuchi M, Kawasaki S and Kakazu T: Criteria for safe hepatic resection. Am J Surg *169(6)*: 589-594, 1995. PMID: 7771622.

11 Lau H, Man K, Fan ST, Yu WC, Lo CM and Wong J: Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. Br J Surg *84(9)*: 1255-1259, 1997. PMID: 9313707.

12 Nakata T, Seki N, Miwa S, Kobayashi A, Soeda J, Nimura Y, Kawasaki S and Miyagawa S: Identification of genes associated with multiple nodules in hepatocellular carcinoma using cDNA microarray; multicentric occurrence or intrahepatic metastasis? Hepatogastroenterology *55(84)*: 865-872, 2008. PMID: 18705285.

13 Li Q, Wang J, Juzi JT, Sun Y, Zheng H, Cui Y, Li H and Hao X: Clonality analysis for multicentric origin and intrahepatic metastasis in recurrent and primary hepatocellular carcinoma. J Gastrointest Surg *12(9)*: 1540-1547, 2008. PMID: 18629593. DOI: 10.1007/s11605-008-0591-y

14 Suzuki K, Hirooka Y, Tsujitani S, Yamane Y, Ikeguchi M and Kaibara N: Relationship between loss of heterozygosity at microsatellite loci and computerized nuclear morphometry in hepatocellular carcinoma. Anticancer Res *20(2B)*: 1257-1262, 2000. PMID: 10810431.

15 Li SP, Wang HY, Li JQ, Zhang CQ, Feng QS, Huang P, Yu XJ, Huang LX, Liang QW and Zeng YX: Genome-wide analyses on loss of heterozygosity in hepatocellular carcinoma in Southern China. J Hepatol *34(6)*: 840-849, 2001. PMID: 11451167.

16 Li S, Wang H and Zhang C: Genome-wide loss of heterozygosity analyses in primary hepatocellular carcinoma. Zhonghua Yi Xue Za Zhi *80(8)*: 577-581, 2000. PMID: 11798820.

17 Pang JZ, Qin LX, Ren N, Hei ZY, Ye QH, Jia WD, Sun BS, Lin GL, Liu DY, Liu YK and Tang ZY: Loss of heterozygosity at D8S298 is a predictor for long-term survival of patients with tumor-node-metastasis stage I of hepatocellular carcinoma. Clin Cancer Res *13(24)*: 7363-7369, 2007. PMID: 18094418. DOI: 10.1158/1078-0432.CCR-07-0593

18 Zhang SH, Cong WM, Xian ZH and Wu MC: Features of micro satellite alterations on chromosome 4 in hepatocellular carcinoma. Zhonghua Gan Zang Bing Za Zhi *12(4)*: 223-226, 2004. PMID: 15099473.

19 Niu Q, Tang ZY, Qin LX, Ma ZC and Zhang LH: Loss of heterozygosity at D14S62 and D14S51 detected by a simple and non-radioactive method in plasma DNA is a potential marker of metastasis and recurrence after curative hepatic resection in hepatocellular carcinoma. Hepatogastroenterology *50(53)*: 1579-1582, 2003. PMID: 14571790.

20 Chen YJ, Yeh SH, Chen JT, Wu CC, Hsu MT, Tsai SF, Chen PJ and Lin CH: Chromosomal changes and clonality relationship between primary and recurrent hepatocellular carcinoma. Gastroenterology *119(2)*: 431-440, 2000. PMID: 10930378.

21 Shao J, Li X and Liu Z: Loss of heterozygosity on chromosomes 17 and 16 in primary hepatocellular carcinomas. Zhonghua Yi Xue Za Zhi *79(6)*: 428-430, 1999. PMID: 11715437.

22 Cong WM, Zhang SH, Xian ZH, Wu WQ and Wu MC: Study on loss of heterozygosity and microsatellite instability in hepatocellular carcinoma. Zhonghua Bing Li Xue Za Zhi *34(2)*: 71-74, 2005. PMID: 15842799.

23 Kawai H, Suda T, Aoyagi Y, Isokawa O, Mita Y, Waguri N, Kuroiwa T, Igarashi M, Tsukada K, Mori S, Shimizu T, Suzuki Y, Abe Y, Takahashi T, Nomoto M and Asakura H: Quantitative evaluation of genomic instability as a possible predictor for development of hepatocellular carcinoma: comparison of loss of heterozygosity and replication error. Hepatology *31(6)*: 1246-1250, 2000. PMID: 10827149. DOI: 10.1053/jhep.2000.7298

24 Joseph CG, Darrah E, Shah AA, Skora AD, Casciola-Rosen LA, Wigley FM, Boin F, Fava A, Thoburn C, Kinde I, Jiao Y, Papadopoulos N, Kinzler KW, Vogelstein B and Rosen A: Association of the autoimmune disease scleroderma with an immunologic response to cancer. Science *343(6167)*: 152-157, 2014. PMID: 24310608. DOI: 10.1126/science.1246886

25 Wooster R, Cleton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BA, von Deimling A and Wiestler OD: Instability of short tandem repeats (microsatellites) in human cancers. Nat Genet *6(2)*: 152-156, 1994. PMID: 8162069. DOI: 10.1038/ng0294-152

26 Mizumoto R, Kawarada Y and Noguchi T: Preoperative estimation of operative risk in liver surgery, with special reference to functional reserve of the remnant liver following major hepatic resection. Jpn J Surg *9(4)*: 343-349, 1979. PMID: 232519.

27 Nagasue N, Inokuchi K, Iwaki A, Yukaya H, Kanashima R, Saku M and Kobayashi M: Remnant liver function during surgery for extensive hepatic resection. Jpn J Surg *9(2)*: 125-131, 1979. PMID: 221710.

28 Poon RT and Fan ST: Evaluation of the new AJCC/UICC staging system for hepatocellular carcinoma after hepatic resection in Chinese patients. Surg Oncol Clin N Am *12(1)*: 35-50, 2003. PMID: 12735128.

29 Le DT, Durham JN and Smith KN: Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science *357(6349)*: 409-413. PMID: 28596308. DOI: 10.1126/ science. aan6733

30 Jin Z and Yoon HH: The promise of PD-1 inhibitors in gastro-esophageal cancers: microsatellite instability *vs*. PD-L1. J Gastrointest Oncol *7(5)*: 771-788, 2016. PMID: 27747091.

31 Furuta M, Ueno M, Fujimoto A, Hayami S, Yasukawa S, Kojima F, Arihiro K, Kawakami Y, Wardell CP, Shiraishi Y, Tanaka H, Nakano K, Maejima K, Sasaki-Oku A, Tokunaga N, Boroevich KA, Abe T, Aikata H, Ohdan H, Gotoh K, Kubo M, Tsunoda T, Miyano S, Chayama K, Yamaue H and Nakagawa H: Whole genome sequencing discriminates hepatocellular carcinoma with intrahepatic metastasis from multi-centric tumors. J Hepatol *66(2)*: 363-373, 2017. PMID: 27742377. DOI: 10.1016/j.jhep. 2016.09.021

32 Qin LX and Tang ZY: The prognostic significance of clinical and pathological features in hepatocellular carcinoma. World J Gastroentero *8(2)*: 193-199, 2002. PMID: 11925590. DOI: 10.3748/wjg.v8.i2.193

33 Wu MC and Shen F: Progress in research of liver surgery in China. World J Gastroenterol *6(6)*: 773-776, 2000. PMID: 11819694. DOI: 10.3748/wjg.v6.i6.773

34 Rabe C, Pilz T, Klostermann C, Berna M, Schild HH, Sauerbruch T and Caselmann WH: Clinical characteristics and outcome of a cohort of 101 patients with hepatocellular carcinoma. World J Gastroenterol *7(2)*: 208-215, 2001. PMID: 11819762. DOI: 10.3748/wjg.v7.i2.208

35 Sithinamsuwan P, Piratvisuth T, Tanomkiat W, Apakupakul N and Tongyoo S: Review of 336 patients with hepatocellular carcinoma at Songklanagarind Hospital. World J Gastroenterol *6(3)*: 339-343, 2000. PMID: 11819593. DOI: 10.3748/wjg. v6.i3.339

36 Makino Y, Yamanoi A, Kimoto T, El-Assal ON, Kohno H and Nagasue N: The influence of perioperative blood transfusion on intrahepatic recurrence after curative resection of hepatocellular carcinoma. Am J Gastroenterol *95(5)*: 1294-1300, 2000. PMID: 10811342. DOI: 10.1111/j.1572-0241.2000.02028.x

37 Toyosaka A, Okamoto E, Mitsunobu M, Oriyama T, Nakao N and Miura K: Pathologic and radiographic studies of intrahepatic metastasis in hepatocellular carcinoma; the role of efferent vessels. HPB Surg *10(2)*: 97-103, 1996. PMID: 9184864.

38 Su M, Li LQ, Peng T, Guo Y, Xiao KY, Shang LM, Xu BH, Li SL, Su ZX and Ye XP: Comparative proteomic approach in differentiating multicentric occurrence and intrahepatic metastasis in multinodular hepatocellular carcinomas. Chin J Cancer *29(1)*: 52-58, 2010. PMID: 20038311.

39 Arii S, Monden K, Niwano M, Furutani M, Mori A, Mizumoto M and Imamura M: Results of surgical treatment for recurrent hepatocellular carcinoma; comparison of outcome among patients with multicentric carcinogenesis, intrahepatic metastasis, and extrahepatic recurrence. J Hepatobiliary Pancreat Surg *5(1)*: 86-92, 1998. PMID: 9683759.

40 Kim JM, Kwon CH, Joh JW, Park JB, Lee JH, Kim SJ, Paik SW and Park CK: Intrahepatic metastasis is more risky than multiple occurrence in hepatocellular carcinoma patients after curative liver resection. Hepatogastroenterology *62(138)*: 399-404, 2015. PMID: 25916071.

41 Li SL, Su M, Peng T, Xiao KY, Shang LM, Xu BH, Su ZX, Ye XP, Peng N, Qin QL, Chen DF, Chen J and Li LQ: Clinicopathologic characteristics and prognoses for multicentric occurrence and intrahepatic metastasis in synchronous multinodular hepatocellular carcinoma patients. Asian Pac J Cancer Prev *14(1)*: 217-223, 2013. PMID: 23534727.

42 Zhou Y, Xu D, Wu L and Li B: Meta-analysis of anatomic resection *versus* nonanatomic resection for hepatocellular carcinoma. Langenbecks Arch Surg *396(7)*: 1109-1117, 2011. PMID: 21476060. DOI: 10.1007/s00423-011-0784-9

43 Chen J, Huang K, Wu J, Zhu H, Shi Y, Wang Y and Zhao G: Survival after anatomic resection *versus* nonanatomic resection for hepatocellular carcinoma: a meta-analysis. Dig Dis Sci *56(6)*: 1626-1633, 2011. PMID: 21082347. DOI: 10.1007/s10620-010-1482-0

Received June 7, 2019
Revised June 27, 2019
Accepted June 28, 2019