表　肝癌に対するラジオ波焼灼療法(RFA)の一般的な適応

肝癌結節から見た適応
1）原則として結節型肝癌
2）腫瘍径 3 cm 以下，腫瘍個数 3 個以下
3）腫瘍が安全に穿刺治療できる部位に存在する
肝予備能から見た適応
1）肝予備能が Child-Pugh A あるいは B
2）穿刺治療が安全に施行できる症例である
・血小板数 5 万以上
・PT 値 50%以上
・コントロール不能の腹水がない

治療効果の確実性と安全性から肝切除，局所療法，肝動脈(化学)塞栓療法[TA(C)E]と順次治療選択を決定する．しかし，実際の臨床においては，合併症の有無，最近増加している高齢者肝癌，あるいは患者が外科手術を強く希望しない場合などの状況に応じて治療選択がなされる場合があるが，あくまでも治療アルゴリズムの原則に則ってインフォームドコンセントを行い，患者の了解にそってRFA 治療が選択されるべきである．

肝癌に対する局所療法は RFA のほかに，MCT，PEI がある．また，RFA や PEI においては TA(C)E との併用により治療効果が高まることが期待されている．これらの治療効果と適応について，『科学的根拠に基づく肝癌診療ガイドライン 2005 年版』で以下に示す通りの推奨がなされている．

PEI
MCT

①PEI 単独治療の良い適応は肝障害度 B の 2 cm 未満の肝細胞癌である．②MCT 単独治療の良い適応は 2～3 cm 以下の肝細胞癌であり，ことに 15 mm 以下では治療効果が良好である．③PEI 治療後の局所再発は RFA や MCT より高率である．④MCT，RFA の合併症の発生頻度は PEI に比して高率であり，この点に留意して治療に臨むべきである．⑤TA(C)E 後あるいは血流遮断下に RFA を行うことにより壊死範囲は拡大するが，予後の改善に関しては今後の検討が必要である．⑥RFA は PEI に比し少ない施行回数で高い完全壊死率が得られ，局所制御能も PEI を凌ぐ．しかし現段階では RFA が PEI より高い生存率をもたらすか否かは不明である．今後，無作為比較試験を実施し，遠隔成績を比較する必要がある．

以上の推奨のうち，推奨⑥に関しては Shiina ら[2]，Lin ら[3]により報告された RFA と PEI，経皮的酢酸注入療法の無作為比較試

験の結果から，肝癌に対する局所療法としてRFAはPEIや経皮的酢酸注入療法よりも先に選択されるべき治療であることが証明された．したがって，現時点では肝癌に対する局所療法としてRFAが第一選択であり，RFAによる合併症の可能性が高い場合や，RFA治療後の追加治療としてPEIは選択されるべきである．安易にPEI単独治療を選択すると治療効果が不十分になる可能性が高くなるので，注意しなければいけない．RFAの安全性については別項で述べられるが，RFAを安全に施行するために穿刺技術の修練や，より安全な穿刺治療に関連する機器を準備することが大切である．

RFAが第一選択

III. RFAの手技

本邦における肝癌のRFAは，経皮的に局所麻酔下で超音波誘導下に施行されていることが多い．超音波診断装置を用いて治療する場合には，①治療の対象とする肝癌結節全体が超音波診断装置で十分に描出される，②穿刺ラインに穿刺の障害となる脈管，胆管が存在しない，③近接する他臓器(胆嚢や腸管，大血管，腎臓など)に障害の及ばない部位に存在する，④その他，前述した安全な治療に必要な条件を満足していることが必要条件である．とくに，肝癌結節の画像診断において，超音波診断像とCTやMRI診断像において腫瘍径に不一致があるときには，単結節周囲増殖型や多結節癒合型などの治療しにくい肝癌結節である可能性を考慮して十分な術前診断をしておくことが大切である．

超音波誘導下

局所麻酔下に経皮RFAを行う場合，以下の手順としている．

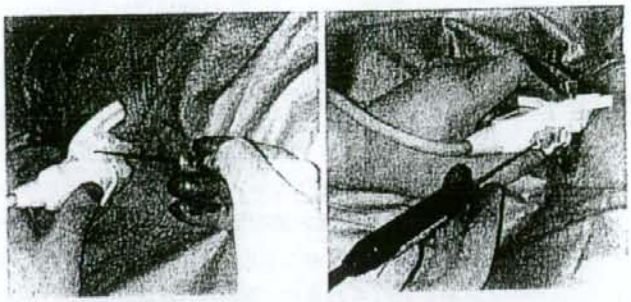
1）術前処置として，硫酸アトロピンとペンタゾシンを投与する．

2）超音波診断装置を用いて通常の呼吸状態で腫瘍が十分に描出され，肝内腫瘍の場合にはもっとも穿刺ラインが短いルートを選択し，肝表面に近い腫瘍の場合には正常肝実質を最初の穿刺ルートとしてとれるラインを選択する．

3）穿刺部位が決定したら皮膚麻酔を行った後に，超音波誘導下に穿刺ラインを局所麻酔する．その後に腫瘍に治療針を挿入するが，このときに穿刺用の誘導針をあらかじめ挿入する施設と，そうでない施設がある．誘導針の使用は針径が大きくなるので治療後の止血に注意が必要である．RFAに用いる治療針の固さが少し弱く，肋間穿刺で場合によっては針が湾曲して目標からずれることがある．筆者はこれを防ぐ目的で肋間用のガイド針を用いている(図

経皮RFAのプロセス

肋間用ガイド針



1a|1b

図1
a：肋間に穿刺針を通す場合，肋間での安定性と，肝穿刺後の直進性を高める目的で肋間用の誘導針を用いる．
b：誘導針の中に穿刺針を通した状態．

1)．この誘導針の使用は結果として肋間動静脈損傷の回避にも役立っているようである．

4）腫瘍を治療針で穿刺した後に，治療を開始するが，焼灼による疼痛を回避するために，焼灼前にペンタゾシン静注あるいはミダゾラム点滴を使用する．患者の呼吸抑制には十分な注意と対策を行う．経験的に2 cmの治療針を用いる場合には局所麻酔でコントロールできることが多いが，3 cmの治療針の場合には疼痛コントロールが不良となることが多く，また多数回の治療にも対応できるように全身麻酔下(筆者は喉頭マスク麻酔を用いている)に治療をしている．

呼吸抑制

5）治療が終了したら治療針を抜去するが，このときには原則として肝実質の止血焼灼を推奨する．止血は肝皮膜下1～2 cmのところで行うが，3 cm針であれば2 cm程度のところを焼灼に伴う針先端ガスの発生が1 cm径になる程度に焼灼している．

後出血

6）治療終了後の回診は3～6時間で行う．治療後の回診は後出血が起こる場合があり必ず行う．

以上が経皮治療の場合の基本的なプロセスであるが，なによりも大切なことは安全に経皮治療が行える症例に行うべきで，それ以外の症例には腹腔鏡下治療，小開胸下治療，胸腔鏡下治療，開腹治療などの安全性を確保した治療法を選択すべきである．

IV. RFAの治療効果判定

造影CT

RFAの治療効果判定は治療後に造影CTで行うのが原則である．治療後2日目あるいは3日目に造影CTを行い治療効果判定を行う(図2)．筆者は全身麻酔下に治療を行うことが多いので，一

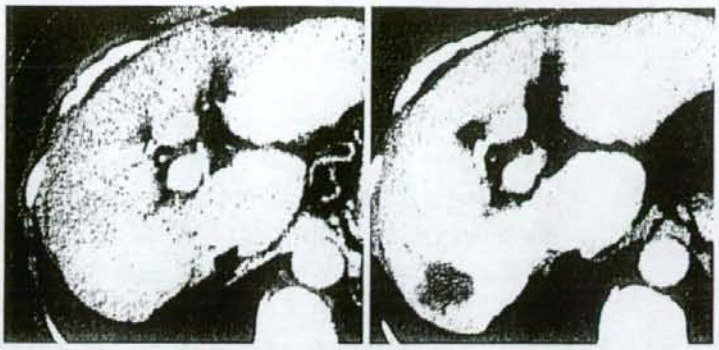


図2
a：肝右葉ドーム下に腫瘍径1.5 cmの濃染を示す肝癌を認める．
b：治療効果判定CT像(造影後期相)．肝癌は十分なマージンをもって焼灼されていることが確認される．

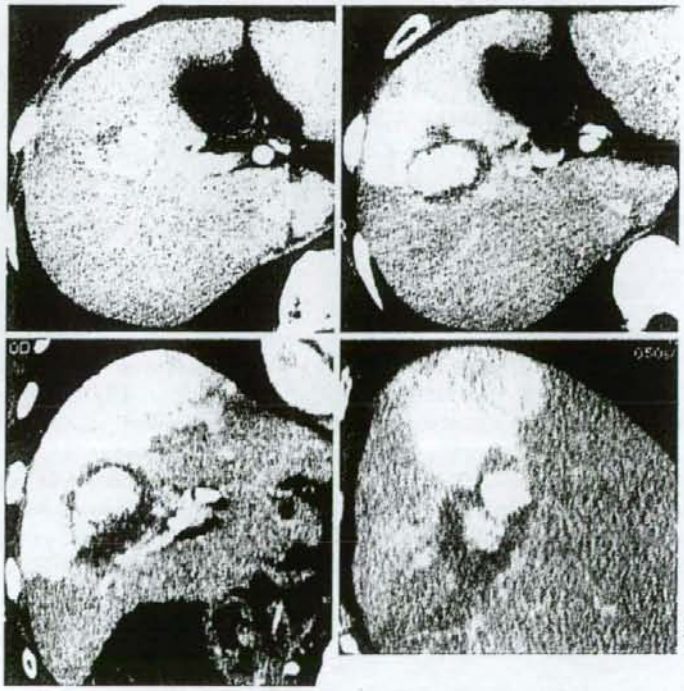


図3
a：肝右葉前区域に腫瘍径3 cmの濃染を示す肝癌を認める．
b：RFA焼灼3日前に肝動脈塞栓術を施行した．RFA治療後3日目の治療効果判定CT像(造影後期相，冠状断)．
c：治療効果判定CT像〔MPRによる矢状断(左右像)〕．
d：治療効果判定CT像〔MPRによる矢状断(前後像)〕．MPR画像による矢状断像と冠状断像を評価することで，腫瘍全体の焼灼が十分かどうか検討することが容易になる．

治療機会で多数回の穿刺で十分な治療を行っているので，すぐに造影CTを施行する．局所麻酔で治療した場合で，再治療の可能性がある場合には造影CTの回数が増加するので，超音波造影剤を用いた治療効果判定を用いてもよいが，あくまでも最終評価は造影CTによって治療終了を決定している．造影CTによる治療効果判定は，できるならMD-CTやヘリカルCTを用いた冠状断像だけでなく，multiplanar reconstruction(MPR)による矢状断像も用いて焼灼した範囲の確認を行って，横方向だけでなく縦方向の焼灼範囲も確認すべきである(図3)．

MPRによる治療効果判定

おわりに

肝癌に対するRFA治療は，その有効性の高さから多くの施設で施行されるようになっている．しかし『科学的根拠に基づく肝癌診療ガイドライン2005年版』にも示されているように，MCT，RFAの合併症の発生頻度はPEIに比して高率であり，すでに治療後の合併症として死亡例も報告されており，安全性に注意して臨むべき治療である．

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Original Article

Effects of lamivudine on outcome after liver resection for hepatocellular carcinoma in patients with active replication of hepatitis B virus

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Aim: Patients with high serum hepatitis B virus (HBV) DNA concentrations are at high risk of tumor recurrence after liver resection for HBV-related hepatocellular carcinoma (HCC).

Methods: Among 24 patients with high serum HBV DNA concentrations who underwent liver resection for HBV-related HCC, postoperative lamivudine therapy was chosen by 14 (lamivudine group). The other 10 patients were controls.

Results: Clinicopathologic findings did not differ between the groups. Tumor-free survival rate after surgery was significantly higher in the lamivudine than the control group ($P = 0.0086$). By univariate analysis, multiple tumors were also a risk factor for a short tumor-free survival. By multivariate analysis, lack of lamivudine therapy and multiple tumors were independent risk factors for a short tumor-free survival. In four patients YMDD mutant viruses were detected after beginning lamivudine administration; in two of them, adefovir dipivoxil was administered because of sustained serum alanine aminotransferase elevations.

Conclusion: Lamivudine therapy improved tumor-free survival rate after curative resection of HBV-related HCC in patients with high serum concentrations of HBV DNA, although careful follow up proved necessary for the detection of YMDD mutant viruses.

Key words: adefovir dipivoxil, hepatitis B virus, hepatocellular carcinoma, lamivudine, liver resection, YMDD mutant virus

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is an etiologic factor for hepatocellular carcinoma (HCC). Although liver resection is an effective treatment for HCC, cancer often recurs even after curative hepatectomy. We previously investigated risk factors for recurrence after resection of HBV-related HCC, finding a high preoperative serum concentration of HBV DNA to be a strong risk factor for HCC recurrence after surgery.[1] On the other hand, the lack of an acute exacerbation of hepatitis and a sustained low serum concentration of HBV DNA after surgery were significantly associated with a low risk of HCC recurrence after resection.[2] We also found substantial serum concentrations of HBV DNA persisting after surgery to be significantly related to short postoperative survival.[2] These findings indicated that biochemical evidence of remission of hepatitis and clearance of viral markers from serum after the operation decrease the likelihood of tumor recurrence.

Recently, a nucleotide analog that inhibits reverse transcriptase, lamivudine, has been developed.[3–5] Lamivudine was found to inhibit the replication of HBV, reduce hepatitis and improve histologic findings in the liver during long-term treatment.[3,6–8] However, lamivudine therapy is not free from problems, such as relapse of hepatitis as a result of the emergence of YMDD mutant viruses.[9–11] Recent studies demonstrated that lamivudine therapy slows the progression of severe liver disease to cirrhosis as well as to HCC.[12,13] However, the effects of lamivudine on outcome after resection of HBV-related HCC are unclear.

In this study we investigated the effects of lamivudine on outcome after resection of HCC in patients with high serum concentrations of HBV DNA.

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METHODS

Patients

FROM NOVEMBER 2000 to October 2005, curative resection of HCC was performed at Osaka City University Hospital in 24 patients seropositive for HB surface antigen (HBsAg) who were negative for antihepatitis C virus antibody and had high serum concentrations of HBV DNA. The patients had not received any lamivudine therapy before the operation. Curative resection was defined as a complete resection of all macroscopically evident tumors. Absence of tumor cells along the parenchymal transection line was confirmed histologically. No remaining tumor was detected in the remnant liver by computed tomography (dynamic study) 3–4 weeks after surgery. The serum concentration of HBV DNA was determined with a transcription-mediated amplification and hybridization protection assay (TMA-HPA) kit, given that serum HBV DNA concentration measured by this method has been shown to be closely related to its hepatitis activity and useful for predicting risk of recurrence after resection of HBV-related HCC.[14,15] In the 24 patients, the serum concentration of HBV DNA was at least 3.7 logarithms of the genome equivalent (LGE) per milliliter, which was considered to represent a high concentration. The patients were told about the previously reported finding that high pre- and postoperative viral concentrations were strong risk factors for HCC recurrence after the operation. Antiviral drug therapeutic effects and side-effects, including the appearance of YMDD mutant viruses, were also explained to the 24 patients. Lamivudine therapy was started in the 14 who then agreed to this therapy and gave their informed consent (lamivudine group), while the control group consisted of the other 10 patients who declined treatment with the drug because of the possibility of adverse events or the necessity of long-term administration of the drug. The 24 patients were followed up until the detection of HCC recurrence or for at least for 6 months in patients without recurrence. The median follow up from operation until the detection of HCC recurrence or the study endpoint (30 April 2006) in this study was 759 days (34–2053). The median follow up for each group was 1117 days (187–2037) for patients receiving lamivudine and 224 days (34–2053) for the controls.

Examination of viral status and active hepatitis

HBsAg and HB envelope antigen (HBeAg) were assayed using an enzyme immunoassay (International Reagents, Kobe, Japan). Anti-hepatitis C virus antibody was assayed using an enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Tokyo, Japan). We used a TMA-HPA kit (Chugai Diagnostics, Tokyo, Japan) to measure serum concentration of HBV DNA. TMA-HPA can quantify 3.7–9.7 LGE/mL (5×10^3–5×10^8 copies/mL). Serum alanine aminotransferase (ALT) activity usually increased immediately following liver resection, then decreased gradually to preoperative activity within 3 weeks. A serum ALT activity at any clinical follow-up assessment (at least every 3 months) of 45 IU/L or less was considered a sustained low ALT activity. The serum concentration of HBV DNA was measured at least twice a year. A serum HBV DNA concentration at any follow up of less than 3.7 LGE/mL was considered a sustained low concentration in the absence of YMDD mutant viruses. YMDD mutant viruses were detected by a reverse hybridization line probe assay (INNO-LiPA HBV DR, Innogenetics, Ghent, Belgium) when serum HBV DNA returned to concentrations above the reference range (≥3.7 LGE/mL) after a decrease to the reference range (<3.7 LGE/mL) with lamivudine therapy.[16,17]

Antiviral treatment

Fourteen patients received lamivudine therapy (100 mg/day), beginning 2 weeks to 2 months after surgery. The period of lamivudine administration was 6 months to 65 months (mean, 32 months). When active hepatitis with a high viral concentration reemerged following proliferation of YMDD mutant viruses, adefovir dipivoxil was administered if patients gave their informed consent. Interferon therapy was carried out in one patient after administration of lamivudine for 6 months, before any appearance of YMDD mutant viruses, to avoid the emergence of YMDD mutant virus.

Pathologic examination

Histologic grading of tumors with respect to differentiation was carried out using the classification of Edmondson and Steiner[18] with certain modifications.[19] When clusters of cancer cells were present in the portal vein, we defined the case as positive for portal invasion. Cancer cells in intracapsular blood vessels, considered branches of the portal vein, also indicated portal vein invasion. When the tumor-free surgical margin was less than 5 mm according to pathologic examination, the margin was defined as positive. Noncancerous hepatic tissues also were examined pathologically. The histologic activity index (HAI) score[20] was used to evaluate the severity of active hepatitis and the degree of fibro-

sis. An HAI score of 0 (based on components 1–3) indicated no activity (grade 0); a score of 1–3, minimal activity (grade 1); a score of 4–8, mild activity (grade 2); a score of 9–12, moderate activity (grade 3); and a score of greater than 12, severe activity (grade 4). The histologic fibrosis score (stage) was determined from component 4 in the HAI scoring. A score of 1 indicated portal fibrous expansion; 2, portal-to-portal septa without architectural distortion; 3, portocentral septa with architectural distortion; and 4, cirrhosis.

Detection of tumor recurrence

Serum α-fetoprotein concentrations were measured every three months. Ultrasonography, computed tomography, magnetic resonance imaging, chest radiography, or a combination of these was performed every three months. When tumor recurrence was suspected on the basis of a tumor marker, radiologic studies, or both, angiography or biopsy was performed to obtain a definitive diagnosis.

Statistics

Student's *t*-test was used to analyze differences in age and tumor size. The Mann–Whitney *U*-test was used to analyze the differences in results of laboratory tests. Fisher's exact test was used to compare categorical data between groups. Tumor-free survival rates were calculated by the Kaplan–Meier method, and the significance of differences in rates between the groups was assessed by the log-rank test. For multivariate analysis, the Cox proportional hazards model with a stepwise method was used. Variables significant at a *P*-value less than 0.1 on univariate analysis were subjected to multivariate analysis. A *P*-value less than 0.05 was considered indicative of significance.

RESULTS

CLINICOPATHOLOGIC FINDINGS IN the lamivudine and control groups are described in Table 1. Age, gender distribution, the proportion of patients with

Table 1 Clinicopathologic findings of patients with and without lamivudine therapy

Findings	Lamivudine therapy		*P*-value
	Yes, $n = 14$	No, $n = 10$	
Age, years, mean ± SD	55 ± 8	55 ± 5	0.993
Gender, men : women	10:4	7:3	>0.999
HBeAg positivity§	11	5	0.204
HBV DNA concentration (LGE/ml)	6.0 ± 1.2	6.0 ± 1.2	0.975
Total bilirubin, mg/dL	0.8 (0.4–1.3)	0.8 (0.4–1.3)	0.656
Albumin, g/dL	3.8 (3.3–4.3)	3.7 (2.6–4.2)	0.426
AST‡, IU/L	44 (35–109)	40 (25–128)	0.578
ALT†, IU/L	53 (25–141)	56 (30–125)	0.558
Child–Pugh classification(A : B)	11:3	8:2	>0.999
α-Fetoprotein, >20 ng/mL	11	7	0.665
Tumor size, cm	2.4 ± 0.7	2.8 ± 1.4	0.329
Multiple tumors	5	5	0.679
Differentiation of main tumor			
Moderately	7	3	0.421
Poorly	7	7	
Portal invasion	4	4	0.673
TMN stage (1:2:3)	2:7:5	3:2:5	0.305
Severity of active hepatitis			
Grade 1, minimal	5	3	>0.999
Grade 2, mild	9	7	
Degree of fibrosis			
Stages 1–3, mild to moderate	8	6	>0.999
Stage 4, cirrhosis	6	4	
Anatomic resection	8	6	>0.999

†ALT, alanine aminotransferase; ‡AST, aspartate aminotransferase; §HBeAg, hepatitis B envelope antigen.
Results of laboratory tests are expressed as medians, with ranges in parentheses.

Table 2 Changes in number of patients with low serum concentration of HBV DNA after surgery

	No. of patients with low serum concentration of HBV DNA (<3.7 LGE/ml)/No. of patients at risk											
	Time after surgery (years)											
	Before	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Control	10/10	1/8	1/4	1/4	1/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Lamivudine	14/14	11/14	8/11	8/11	6/11	4/8	4/8	4/7	4/6	4/5	3/4	1/1

$p = 0.0028$.

HBeAg positivity, viral load, the results of laboratory tests, and Child–Pugh classification[21] did not differ between groups. Type of resection, tumor size, tumor number, differentiation of main tumor, prevalence of portal invasion, cancer stage according to UICC classification,[22] severity of active hepatitis as well as degree of hepatic fibrosis in non-cancerous hepatic tissue showed no difference between groups. No patients had portal invasion detected by preoperative medical imagings. The changes in the number of patients with low serum concentration of HBV DNA and low ALT activity after the operation until the detection of HCC recurrence or the study endpoint in both groups are shown in Tables 2 and 3, respectively. In one patient in the lamivudine group, serum HBeAg changed from positive to negative after the operation.

The tumor-free survival rate was significantly higher in the lamivudine group than in the control group (Fig. 1, $P = 0.0086$). In one patient in the lamivudine group and four patients in the control group, multiple recurrent tumors were detected in the remnant liver. In one patient in the lamivudine group and in one patient in the control group, a single recurrent tumor was detected. By univariate analysis using preoperative factors, multiple tumors was also a risk factor for short tumor-free survival ($P = 0.0036$). By multivariate analysis, lack of lamivudine therapy and multiple tumors were independent risk factors for short tumor-free survival (risk ratio, 18.306; 95% confidence interval, 1.707–196.327; $P = 0.0163$, 16.949; 1.686–166.667; $P = 0.0162$, respectively). Thus, the lack of lamivudine therapy was an independent risk factor for recurrence.

A sustained low serum concentration of HBV DNA was observed in no patient in the control group and in eight patients in the lamivudine group. Although HCC did not recur in the eight patients with sustained low serum concentrations of HBV DNA, HCC recurred in seven of 16 patients with high serum concentrations of HBV DNA (Fig. 2). The proportion of patients with multiple tumors was significantly higher in the seven patients than in nine patients without recurrence. In the lamivudine group, HCC recurred in two patients, who had sustained high serum concentrations of HBV DNA. Sustained normal ALT activity was observed in five patients in the control group and in 10 patients in the lamivudine group. In one patient in the control group, ulsodeoxycholic acid was administered because serum ALT activity was more than 90 IU/L (twice the reference range). In four other patients, no treatment was performed because ALT activity was less than 90 IU/L. Although HCC recurred in two of the 15 patients with sustained normal ALT activity, HCC recurred in five of nine patients with high serum ALT activity (Fig. 3, $P = 0.0346$).

Although three patients in the control group died of HCC recurrence, no patients died in the lamivudine group.

In four patients, YMDD mutant viruses were detected at 10, 11, 21 and 43 months after beginning administration of lamivudine and ALT activity increased in three of the four patients. In two of the three patients with sustained high ALT activity, adefovir dipirvoxil was administered. In one patient, YMDD mutant virus was detected when the HCC recurrence was detected. In the three other patients with YMDD mutant viruses, HCC did not recur.

Table 3 Change in number of patients with low alanine aminotransferase activity after surgery

	No. of patients with low alanine aminotransferase activity (≤45 IU/l)/No. of patients at risk											
	Time after surgery (years)											
	Before	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Control	2/10	5/8	2/4	2/4	2/2	1/1	1/1	1/1	1/1	1/1	0/1	0/1
Lamivudine	6/14	10/14	7/11	7/11	6/11	5/8	5/8	5/7	6/6	5/5	2/4	1/1

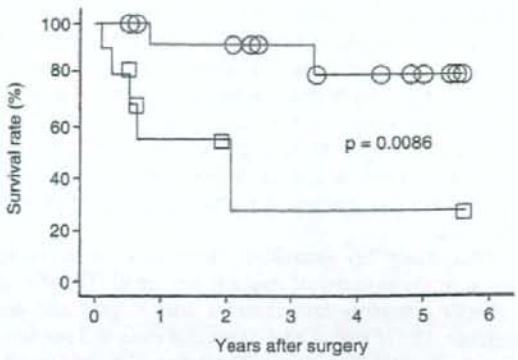

Figure 1 Tumor-free survival rates in lamivudine and control groups. (○), 14 patients with postoperative lamivudine therapy; (□), 10 patients without postoperative lamivudine therapy.

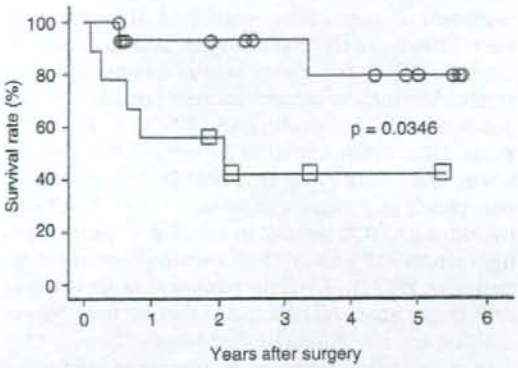

Figure 3 Tumor-free survival rates in patients with and without sustained low serum alanine aminotransferase activity. (○), 15 patients with sustained low serum alanine aminotransferase activity; (□), nine patients with high serum alanine aminotransferase activity.

DISCUSSION

THIS STUDY SHOWED that lamivudine therapy improved the tumor-free survival rate after liver resection for HCC in patients with a high serum concentration of HBV DNA.

This study did not have a randomized prospective design. A well-designed prospective study is difficult to conduct because lamivudine therapy already has been developed as a treatment for chronic hepatitis B and is presently used in clinical practice.[3–8] Despite its status as an established treatment for this form of hepatitis, whether lamivudine therapy is effective for the suppression of tumor recurrence after HBV-related HCC has not been determined. We have reported high preoperative viral concentration and sustained high viral concentration after liver resection to be risk factors for HCC recurrence,[1,2] defining a high-risk patient group. Of 24 patients presently studied, 14 chose lamivudine therapy after explanation of these previous results of liver resection for HBV-related HCC, as well as likely advantages and disadvantages of lamivudine in such a high-risk group. As shown in Table 1, no difference in background characteristics was evident between the lamivudine and control groups. The tumor-free survival rate was significantly higher in the lamivudine group than in the control group, with the absence of lamivudine therapy being an independent risk factor for short tumor-free survival. Thus, this study showed that lamivudine therapy suppressed tumor recurrence after curative resection in these HBV-positive patients.

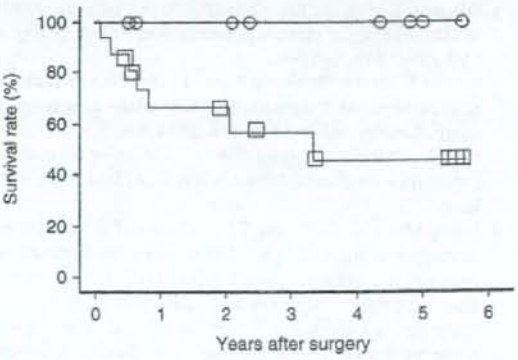

Figure 2 Tumor-free survival rates in patients with and without sustained low serum concentrations of HBV DNA. (○), eight patients with sustained low serum concentration of HBV DNA; (□), 16 patients with high serum concentrations of HBV DNA.

Recurrences after surgery include both intrahepatic metastases from the primary HCC and newly developed HCC (multicentric carcinogenesis). Although the mechanism by which lamivudine therapy suppressed tumor recurrence after surgery was not determined in this investigation, one likely factor underlying the low recurrence rate in the lamivudine group would be a decrease in multicentric carcinogenesis after surgery. Regeneration of hepatocytes amid a background of necrosis during active hepatitis may directly induce DNA mutation, as well as chromosomal rearrangement resulting in genetic instability. Sustained high serum concentrations of HBV and

persistent or intermittent elevation of ALT activity have been linked closely to carcinogenesis in patients with hepatitis B.[23–25] Two recent studies demonstrated that lamivudine therapy reduced the development of HCC in patients with chronic hepatitis B.[12,13] In the present study, HCC did not recur in patients with a sustained low serum concentration of HBV DNA. HCC recurred in only two of 15 patients with sustained normal ALT activity, although HCC recurred in five of nine patients with high serum ALT activity. Thus, sustained serum concentration of HBV DNA and the remission of active hepatitis brought about by lamivudine therapy may decrease multicentric carcinogenesis after surgery.

In our previous study, the percentage of intrahepatic metastases from similarly differentiated main tumors tended to be higher in patients with a high viral concentration than in those with a low viral concentration.[1] HCC thus may behave more aggressively in patients with a high viral concentration, even when histologic differentiation of the tumor is the same; a decrease in viral concentration with lamivudine therapy thus may affect important biologic characteristics of HCC, although lamivudine itself does not have anticancer effects. For example, active hepatitis induces upregulation of adhesion molecules on cells lining hepatic sinusoids, which may enhance the likelihood of metastasis.[26] Remission of active hepatitis in response to lamivudine therapy thus may decrease metastatic potential.

Long-term lamivudine administration is associated with an increasing rate of emergence of drug-resistant viral strains.[9–11] YMDD mutant viruses appeared in four patients in this series. YMDD mutant viruses often cause flare-ups of ALT activity and hepatic decompensation in patients with cirrhosis. In fact, ALT activity increased above the reference range in three of the four patients with YMDD mutant virus. Adefovir dipivoxil was administered in two of the three patients with high ALT activity. In addition to hepatitis exacerbations, viral breakthrough itself may increase the likelihood of the development of HCC,[27] a particular risk in patients with cirrhosis and YMDD mutations.[28] Although HCC did not recur after the appearance of YMDD mutant virus in this series, early identification of YMDD mutant viruses is important. When such mutant viruses are identified, close follow up is necessary to prevent hepatic failure and recurrence of HCC. Several investigators have reported that interferon therapy reduced the incidence of HCC,[29–32] although some others found no decrease.[33,34] Recently, Sun *et al.* reported that interferon therapy improved the overall survival of patients with HBV-related HCC after curative resection, probably by postponing recurrence.[35] Lamivudine appears to be superior to interferon in delaying HCC development.[36] In our series, interferon was administered after lamivudine therapy for 6 months in one patient, who then showed a decreased serum concentration of HBV DNA and ALT activity without appearance of YMDD mutant viruses. A combination of lamivudine and interferon therefore may represent a useful alternative treatment regimen.

This study fell somewhat short of conclusiveness because the number of patients was small. The effects of HBV genotypes on recurrence after surgery also are unclear. The effects of YMDD mutant virus and adefovir dipivoxil on HCC recurrence also should be evaluated. A larger investigation is necessary to clarify the effects of and indications for lamivudine.

In conclusion, lamivudine therapy improved the tumor-free survival rate after curative resection of HBV-related HCC in patients with a high serum concentration of HBV DNA, although careful follow up was necessary for timely detection of YMDD mutant viruses to minimize their adverse effects.

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Clinicopathological implications of immunohistochemically demonstrated mucin core protein expression in hepatocellular carcinoma

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Abstract
Methods. We examined the expression of mucin core protein 1 (MUC1) immunohistochemically in 186 surgical specimens of histopathologically nonmucinous hepatocellular carcinoma (HCC) and compared the clinicopathological features in patients with MUC1-positive HCC (MUC1-positive group) with those in patients with MUC1-negative HCC (MUC1-negative group).
Results. MUC1 immunoreactively was present in 85 of the 186 HCCs. Of the clinicopathological variables examined, the serum concentration of α-fetoprotein, tumor differentiation, bile duct invasion, lymph node metastasis, and cytokeratin 19 expression exhibited significant associations with MUC1 expression. Although cumulative and tumor-free survival rates were not different between the two groups, the percentage of patients with first recurrence of HCC in distant organs (distant metastasis) within 2 years after surgery was significantly higher in the MUC1-positive group than in the MUC1-negative group ($P = 0.0104$). The risk ratio of MUC1 positivity for this type of distant metastasis was 3.156 (95% confidence interval, 1.064–9.358).
Conclusions. In patients with MUC1-positive HCC, careful follow-up is necessary, not only for intrahepatic recurrence but also for distant metastasis, after the resection of primary HCC.

Key words Hepatocellular carcinoma · Mucin core protein · Distant metastasis · Lymph node metastasis

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Introduction

We previously reported that, apart from cases of combined hepatocellular carcinoma (HCC) and intrahepatic cholangiocellular carcinoma (ICC), some HCCs expressed bile-duct associated cytokeratins (CKs) 7 and 19,[1] and that patients with such HCCs had relatively poor survivals.[2] Although CKs 7 and 19 are commonly expressed by ICC,[3–7] these biliary markers are also sometimes expressed in typical HCC.[1,8–10] We hypothesized that HCC expressing CKs 7 and 19 might have some of the characteristics of ICC favoring invasion and metastasis.[2]

Mucin core protein 1 (MUC1) is a type I transmembrane protein with a large number of tandem repeats consisting of 20 amino acids, and is a member of a family of mucin glycoproteins that are characterized by high carbohydrate content, O-linked oligosaccharides, high molecular weight, and an amino-acid composition rich in serine, threonine, proline, and glycine.[11,12] In normal tissues, the expression of MUC1, a constituent of mucins, as well as the expression of CKs 7 and 19, commonly identifies tissue as ductular epithelium.[11–14] In normal human liver, intrahepatic bile duct epithelial cells express MUC1, while hepatocytes do not,[15] as is true of CK7 and CK19 as well. Some adenocarcinoma cells express MUC1,[14,16–20] and this expression in adenocarcinomas has been associated with poor survival outcome.[16,17,21–23] These findings suggest that the expression of not only CKs 7 and 19 but also MUC1 could be a useful indicator of malignant potential in primary liver cancer.[15,19,24,25] We therefore examined the association of MUC1 expression in histologically typical HCC with clinicopathological features, including the expression of CKs 7 and 19 and postoperative outcome.

Patients and methods

Patients

Between 1995 and 2002, we performed hepatic resections for primary HCC in 186 patients at Osaka City University Hospital. The patients were followed for at least 2 years after surgery, or until death. We analyzed their clinical records and the 186 liver tumor resection specimens, which were fixed in 10% formalin, embedded in paraffin, and sectioned at 3-μm intervals. After the specimens were stained with hematoxylin and eosin and by the periodic acid-Schiff method, including diastase digestion (diastase-PAS), two experienced pathologists diagnosed the 186 tumors. Microscopically, HCC had a typical histological appearance, including eosinophilic cytoplasm and a thin or thick trabecular architecture mimicking that of the liver, without diastase-PAS staining in tumor cells. In this study, we excluded the mixed type of HCC and ICC.

The 186 patients consisted of 154 men and 32 women, with an age range of 30 to 77 years (mean, 63 years). Antibody to hepatitis C virus was positive in 133 patients (72%); antibody to hepatitis B surface antigen was positive in 27 (15%); and antibody to hepatitis B core was positive in 119 (64%). Preoperative serum concentrations of total bilirubin, albumin, α-fetoprotein (AFP; reference range, 20 ng/ml or less), and a soluble fragment of CK 19 in serum (CYFRA 21-1;[26,27] reference range, <2.0 ng/ml), as well as prothrombin time, were measured. Patients were examined preoperatively by ultrasonography, computed tomography (CT), and angiography. CT during arteriography and CT during arterioportography were performed, if possible. Intraoperative ultrasonography was performed in all patients. Serum AFP concentration was measured every 3 months after surgery. Ultrasonography, CT, magnetic resonance imaging, chest radiography, or combinations of these were performed every 3 months. When tumor recurrence was suspected on the basis of tumor marker examination, radiological studies, or combinations of these, angiography or biopsy under ultrasonographic guidance was performed to obtain a definitive diagnosis. The presence of bone metastases was examined by scintigraphy using ^{99m}Tc diphosphonate.

Pathology examination

The diameters of resected tumors were measured in surgical specimens prior to fixation in 10% formalin. The following histopathological factors were examined in hematoxylin-and-eosin-stained sections: differentiation of HCC, formation of pseudoglandular structures, microscopic intrahepatic metastasis, intraluminal invasion of vessels or bile ducts, metastasis to lymph nodes, activity of hepatitis, and degree of hepatic fibrosis. When multiple lesions were demonstrated, the largest nodule was identified as the principal tumor nodule and others were considered satellite lesions. Satellite lesions were classified as representing multicentric occurrence of HCC when at least one tumor consisting of well-differentiated HCC grew in a replacement growth pattern, with hepatic structures maintained, and with a moderately or poorly differentiated main tumor elsewhere.[28,29] Multiple HCCs that did not meet these criteria were assumed to be intrahepatic metastases originating from a main tumor.

The pathologists assessed the above pathological findings for HCC according to the "Classification of primary liver cancer", as well as assessing chronic hepatitis in noncancerous areas, according to the grades of inflammation and stages of fibrosis defined by Desmet et al.[30] and Knodell et al.[31]

Immunohistochemistry

Immunohistochemical staining was performed with antibodies to human hepatocyte (HC), CK7, CK19, MUC1, using a modified avidin-biotin complex method (Envision system kit; Dako Japan, Kyoto, Japan).[32,33] The primary antibodies were anti-HC antibody (mouse monoclonal; Dako Japan), anti-human CK7 antibody (mouse monoclonal; Dako Japan), anti-human CK19 antibody (mouse monoclonal; Dako Japan), and anti-human MUC1 antibody (mouse monoclonal; Fujisawa Pharmaceutical, Tokyo, Japan). A normal liver specimen was used as the control specimen. Bile ductules in Glisson's sheath were used as positive controls for CK7, CK19, and MUC1 and as a negative control for HC, while normal hepatocytes in the control specimen were used as a positive control for HC, and as negative controls for CK7, CK19, and MUC1. Examinations of immunohistochemical reactions using the ENVISION kit were performed according to the recommendations of the manufacturer. Briefly, after antigen-antibody reaction between the surgical specimens and the first antibodies, the first antibodies reacted with a second antibody connected to polymeric conjugates of dextran backbone, and the final immunoreactive complexes were stained with diaminobenzidene hydrochloride.[32,33]

Antibodies to HC, CK7, and CK19 were diluted 1:100 with buffer (Dako Japan), while MUC1 was diluted 1:300. HC, CK7, and CK19 antibodies were incubated with the specimens at room temperature for 60 min, while MUC1 incubation was carried out overnight at room temperature. With the examination of ten fields, at ×200, when 10% or more of the tumor cells were immunohistochemically stained with CK7, CK19, and MUC1, findings were considered positive. Theo-

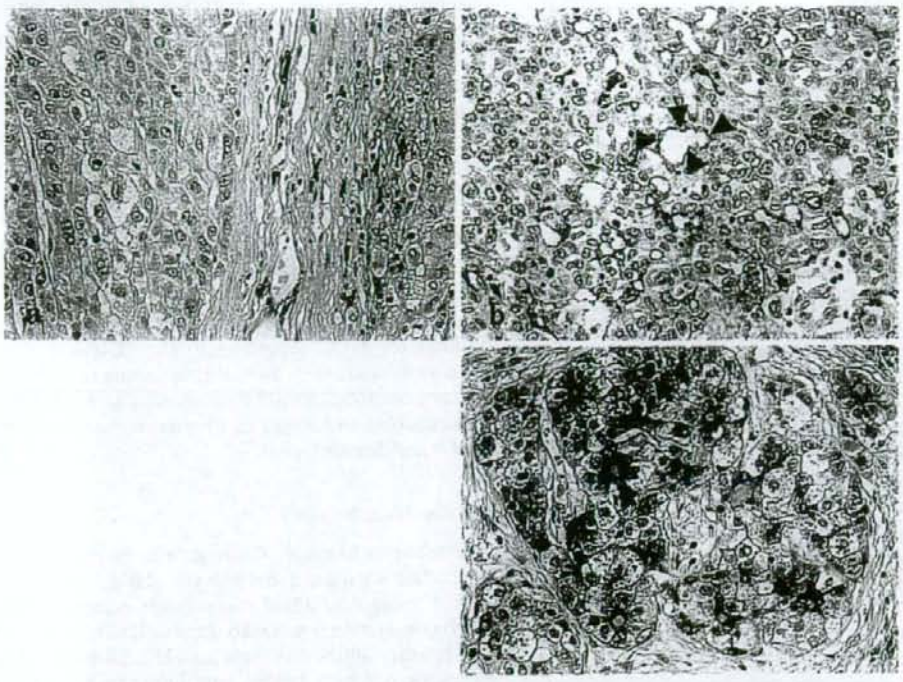

Fig. 1. Microscopic findings for mucin core protein 1 (MUC1)-negative (**a**) and -positive (**b**, **c**) hepatocellular carcinomas. **a** Cancer cells are stained for MUC1, while bile duct epithelial cells are stained for MUC1. **b** Luminal surfaces (*arrowheads*) and **c** luminal surfaces and cytoplasm (*arrows*) of cancer cells are stained for MUC1

retically, the staining of HCC cells with CK7, CK19, and MUC1 in even small amounts could be a strange finding, because it would suggest that the HCC originated from hepatocytes. Therefore, 10% of cells stained with CK7, 19, and MUC1 was regarded as the positive threshold, although this was a small amount. The immunohistochemical localization of MUC1 in HCC was classified as either luminal or lumial-with-cytoplasmic type. The two pathologists judged these immunohistochemical findings by the double-check method.

This study was conducted in accordance with the Helsinki Declaration and the guidelines of the Ethics Committee at our institution. We obtained informed consent from each patient, and followed the patients' courses and postoperative survival.

Statistical analysis

Student's *t*-test was used to examine differences in age and tumor size. The Mann-Whitney *U*-test was used to examine differences in the laboratory test results. The Fisher exact test or the χ^2 test was used to compare categorical data between groups. Tumor-free and cumulative survival rates were calculated by the Kaplan-Meier method, and differences in survival were determined by the log-rank test. Odds ratios were used to estimate relative risks for distant metastasis after liver resection for HCC. For univariate analysis, logistic regression analysis was used. For multivariate analysis, multiple logistic regression analysis was used. The variables chosen were age; sex; serological viral results; laboratory test results; tumor size; tumor differentiation; intrahepatic metastasis; vascular invasion; bile duct invasion; results of immunohistochemical staining with MUC1, CK7, and CK19; activity of hepatitis; degree of hepatic fibrosis; and type of resection (anatomic or nonanatomic). *P* values less than 0.05 were considered significant.

Results

Immunohistochemically, 85 HCCs (46%) were positive for MUC1. Staining for MUC1 showed 54 luminal

Table 1. Clinicopathological findings in patients with hepatocellular carcinomas with and without MUC1 expression

Variables	MUC1 expression		*P* value
	Positive ($n = 85$)	Negative ($n = 101$)	
Age, years, mean ± SD	60.9 ± 9.1	64.9 ± 6.3	0.0005
Sex, male : female	67 : 18	87 : 14	0.188
Laboratory data			
Anti-HCV-positive	58	75	0.365
HBsAg-positive	17	10	0.0514
Anti-HBc-positive	60	59	0.141
AST, IU/l	53 (32, 100)	56 (32, 96)	0.877
ALT, IU/l	59 (27, 113)	62 (27, 106)	0.665
Total bilirubin, mg/dl	0.8 (0.5, 1.3)	0.8 (0.6, 1.3)	0.542
Albumin, g/dl	3.6 (3.1, 4.2)	3.7 (3.3, 4.1)	0.231
Platelet count, $10^4/\mu l$	13.9 (7.0, 24.9)	14.2 (8.3, 24.2)	0.532
α-Fetoprotein, >20 ng/ml	64	37	0.0001
CYFRA 21-1, >2.0 ng/ml	26	26	0.934
Pathology			
Noncancerous areas			
HAI score			
Grade 0–2	72	96	0.0175
3–4	13	5	
Stage 0–3	46	57	0.751
4 (Cirrhosis)	39	44	
Cancers			
Tumor size, cm, mean ± SD	4.2 ± 2.9	4.0 ± 3.3	0.698
Tumor differentiation			
Good	1	14	0.0001
Moderate	44	64	
Poor	40	23	
Intrahepatic metastasis	21	32	0.294
Portal invasion	35	31	0.137
Hepatic vein invasion	3	4	0.878
Bile duct invasion	6	0	0.0066
Lymph node metastasis	4	0	0.0419
Pesudoglandular arrangement	33	31	0.286
Cytokeratin 19-positive	14	3	0.0015
Cytokeratin 7-positive	42	43	0.366

Numbers followed by values in parentheses represent means (and ranges with 10th and 90th percentiles). Numbers standing alone are numbers of patients.
HCV, hepatitic C virus; HBsAg, hepatitic B surface antigen; HBc, hepatitis B core; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CYFRA21-1, soluble fragments of cytokeratin 19 in serum; HAI, histological activity index

and 31 luminal-with-cytoplasmic types (see Fig. 1 for representative findings). The clinicopathological features of the patients are shown in Table 1. Patients with MUC1-positive HCC (MUC1-positive group) were younger than those with MUC1-negative HCC (MUC1-negative group). The percentage of patients with elevated AFP was significantly higher in the MUC1-positive group than that in the MUC1-negative group. The percentage of patients with poorly differentiated HCC was significantly higher in the MUC1-positive group than that in the MUC1-negative group. All six tumors with bile duct invasion were positive for MUC1, representing a significant association. The percentage of patients with lymph node metastasis was significantly higher in the MUC1-positive group than that in the MUC1-negative group. A significant relationship was also observed between the expression of MUC1 and the expression of CK19.

Cumulative and tumor-free survival rates did not differ between the groups (Figs. 2 and 3). Within 2 years after surgery, in the early postoperative phase, tumor recurrence developed in 46 patients in the MUC1-negative group and in 35 patients in the MUC1-positive group. In 5 of the 46 patients in the MUC1-negative group and 12 of the 35 patients in the MUC1-positive group, the site of first recurrence was distant organs, such as lungs, bones, brain, and/or lymph nodes, with and without intrahepatic recurrence. Lymph node metastasis developed in 3 of the 12 patients in the MUC1-positive group. The percentage of

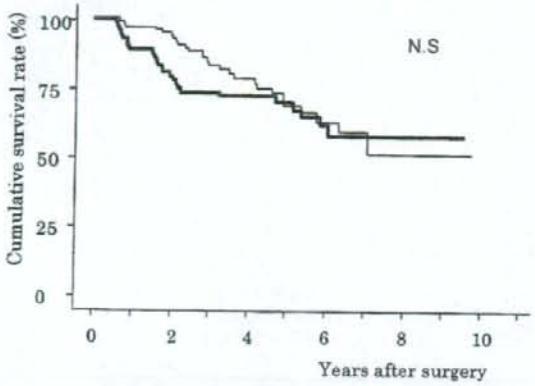

Fig. 2. Cumulative survival rates of patients in the MUC1-positive group (*thick line*) and the MUC1-negative group (*thin line*). *N.S.*, Not significant

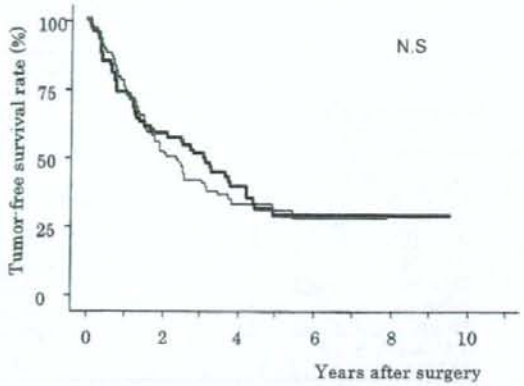

Fig. 3. Tumor-free survival rates of patients in the MUC1-positive group (*thick line*) and the MUC1-negative group (*thin line*)

Table 2. Risk ratios for distant metastasis within 2 years after liver resection for hepatocellular carcinoma (univariate analysis)

Variables	Risk ratio	95% Confidence interval	*P* value
Tumor size (4cm or more)	2.883	1.042–7.976	0.0414
Intrahepatic metastasis	4.186	1.500–11.680	0.0062
Portal invasion	7.113	2.214–22.848	0.0010
Hepatic vein invasion	17.026	3.437–84.328	0.0005
MUC1 positivity	3.156	1.064–9.358	0.0382

patients with such distant metastasis was significantly higher in the MUC1-positive group than in the MUC1-negative group ($P = 0.0104$).

The relative risk of distant metastasis as the first recurrence within 2 years after surgery was evaluated. On univariate analysis, larger tumor (>4cm in diameter), intrahepatic metastasis, portal invasion, hepatic vein invasion, and MUC1 positivity were significant risk factors for such distant metastasis (Table 2). On multivariate analysis, portal invasion (adjusted risk ratio [RR], 4.509; 95% confidence interval [CI], 1.216–16.722; $P = 0.0243$), hepatic vein invasion (RR, 9.324; 95% CI, 1.259–69.060; $P = 0.0289$), and MUC1 positivity (RR, 3.694; 95% CI, 1.064–12.822; $P = 0.0396$) were independent risk factors for such distant metastasis.

Discussion

In this study, 85 HCCs (46%) were immunohistochemically positive for MUC1. Significant relationships were noted between MUC1 positivity and CK 19 positivity, elevations of AFP, poor differentiation, bile duct invasion, and lymph node metastasis. The percentage of patients with distant metastasis in the early postoperative phase was significantly higher in the MUC1-positive group than that in the MUC1-negative group. Nakamori et al.[21] reported that mature MUC1 mucins may represent a phenotype correlated with tumor progression to metastatic stages, possibly making MUC1 a useful marker for the likely progression of human colorectal carcinoma, while Yonezawa et al.[17] suggested that difference in the expression of MUC1 is a useful prognostic indicator of malignant potential in ICC. The correlations we found between MUC1 positivity and CK19 positivity, bile duct invasion, and lymph node metastasis suggest that such HCCs have characteristics of adenocarcinoma, including ICC. Typical HCCs usually invade the portal vein first, spread via the portal vein, and then form an intrahepatic metastasis. On the other hand, ICC and combined/mixed hepatocellular and cholangiocellular carcinoma (mixed HCC) are reactive for CKs 7 and 19 and also form lymph node metastases and extrahepatic metastases (distant metastases).[34-41] Several investigators have reported that the overexpression of mucin on the membranes of cultured cells inhibits tumor-cell aggregation and also inhibits interactions with cytotoxic lymphocytes, probably reflecting the extended chemical structure of mucin.[42,43] Such properties of mucin could influence at least two

important steps of the metastatic process: release of cells from the tumor, and their escape from immune surveillance.[42,44–47] The characteristics of adenocarcinoma, presumably, were responsible for the rapid development of distant metastasis after the resection of MUC1-positive HCC.

The present findings of AFP elevation and poor differentiation suggest the reversion of HCC toward a hepatic progenitor cell phenotype, as is also true for the presence of MUC1 and CK19 in HCC with a typical appearance.[48–50] This is another possible reason for the rapid development of distant metastasis.

In this study, the tumor-free and cumulative survival rates did not differ between the MUC1-positive and -negative groups. A high concentration of AFP, large tumor, moderately or poorly differentiated HCC, intrahepatic metastasis, portal invasion, and hepatic vein invasion have been reported as prognostic factors for HCC.[51–55] Patients with a high concentration of AFP, large tumor, moderately or poorly differentiated HCC, and portal invasion were predominant in our MUC1-positive group, whereas patients with intraheatic metastasis or hepatic vein invasion were predominant in the MUC1-negative group; the various prognostic factors in the two groups resulted in smilar survival curves. Bile duct invasion was not a risk factor for tumor recurrence in this study.

On univariate analysis, we found that the risk factors for distant metastasis in the early postoperative phase were large tumor size, intrahepatic metastasis, portal invasion, hepatic vein invasion, and MUC1 positivity. On multivariate analysis, portal invasion, hepatic vein invasion, and MUC1 positivity were independent risk factors. We have already reported that the frequency of extrahepatic metastasis within 2 years after surgery was significantly higher in patients with CK 19-positive HCC than in those with CK-19 negative HCC.[2] In the present study, a significant relationship was noted between the expression of MUC1 and the expression of CK 19. Although lymph nodes were the preferred site of distant metastasis after surgery in patients with CK-19-positive HCC, the frequency of lymph node metastasis after surgery was low in the MUC1-positve group. In patients with MUC1-positive HCC, distant organs, including lungs, bone, and brain, were the main sites of distant metastasis. These findings indicate that, in patients with MUC1-positive HCC, after surgery, careful follow-up for distant organ metastasis, as well as intrahepatic metastasis, is important, even if patients have HCC without vascular invasion.

In conclusion, the present study indicated that about 40% of HCCs expressed MUC1 and that patients with MUC1-positive HCC were at risk for distant metastasis in the early postoperative phase. Thus, in patients with MUC1-positive HCC, careful follow-up is necessary, not only for intrahepatic recurrence but also for distant metastasis, after resection of the primary HCC. Further study of MUC1-positive HCC is necessary, because the number of patients in this study was too small to obtain definitive conclusions.

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Clinical significance of serum cytokeratin-19 fragment (CYFRA 21-1) in hepatocellular carcinoma

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Abstract

Background/Purpose. CYFRA 21-1, a soluble fragment of cytokeratin 19, is increased in serum in some patients with hepatocellular carcinoma, but the clinical significance of this increase is still unknown.

Methods. Serum concentrations of CYFRA 21-1 were measured in 240 patients with hepatocellular carcinoma prior to hepatic resection. The relationships between serum CYFRA 21-1 concentrations and clinicopathologic features were analyzed.

Results. The sensitivity of CYFRA 21-1 as a test for hepatocellular carcinoma was 18.8%. Serum CYFRA 21-1 was significantly higher in patients with portal vein tumor thrombus, and serum CYFRA 21-1 increased with the progression of portal vein tumor thrombus. Tumor size was related to serum CYFRA 21-1, but there were no significant correlations between serum CYFRA 21-1 concentrations and tumor differentiation or number of tumors. Although patients with stage IV tumor had significantly higher CYFRA 21-1 concentrations than those with stages I, II, and III, CYFRA 21-1 was not associated with postoperative prognosis.

Conclusions. Although high concentrations of CYFRA 21-1 were often detected in patients with a tumor diameter greater than 5 cm or tumor thrombus in the major portal vein, CYFRA 21-1 is not a useful diagnostic tool for hepatocellular carcinoma because of its low sensitivity.

Key words Hepatocellular carcinoma · Cytokeratin 19 · CYFRA 21-1 · Portal thrombus

Introduction

Surveillance of high-risk patients with hepatitis B or C virus (HBV or HCV) infection, based on the measurement of serum alpha-fetoprotein (AFP) and abdominal ultrasonography, has been recommended for the early detection of hepatocellular carcinoma (HCC).[1,2] Measurements of plasma des-gamma-carboxy prothrombin (DCP), a protein induced by vitamin K absence or antagonist (PIVKA) II, can increase sensitivity for the diagnosis of HCC; high DCP levels were observed more often in patients with low AFP-producing HCC.[3–5]

Serum carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 are commonly used in combination for the diagnosis of intrahepatic cholangiocarcinoma (ICC) and for follow-up,[6–8] although insufficient sensitivity and specificity have been a problem.[9,10] Our recent study evaluated serum cytokeratin (CK) 19 fragment (CYFRA 21-1) concentrations in 187 patients with primary liver cancer (164 with HCC and 23 with ICC) and 87 patients with benign liver diseases.[10] The study identified serum CYFRA 21-1 as a useful diagnostic test for ICC because of its outstanding sensitivity (87.0%), whereas only 28 of 164 patients (17.1%) with HCC had high concentrations of serum CYFRA 21-1.[10] Some investigators have also reported high serum CYFRA 21-1 levels in patients with HCC.[11–13] Recently, Wu et al.[14] demonstrated the expression of mRNA for CK19 in three of five HCC cell lines which synthesize CK19 protein and release CYFRA 21-1. Little is known, however, about the clinical significance of the serum level of CYFRA 21-1 in HCC patients. Therefore, we examined the relationship between the serum level of CYFRA 21-1 and clinocopathologic features in patients who underwent hepatic resection for HCC.

Subjects and methods

Patients

This study was performed using consecutively obtained samples from 240 patients with HCC. Serum samples were collected just before surgery and were stored at

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