P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• LIVER CANCER •

# Does surgical resection of hepatocellular carcinoma accelerate cancer dissemination?

I-Shyan Sheen, Kuo-Shyang Jeng, Shou-Chuan Shih, Po-Chuan Wang, Wen-Hsiung Chang, Horng-Yuan Wang, Li-Rung Shyung, Shee-Chan Lin, Chin-Roa Kao, Yi-Chun Tsai, Tsu-Yen Wu

**I-Shyan Sheen,** Divisions of Hepatogastroenterology, Chang Gung Memorial Hospital, Taipei, Taiwan, China
**Kuo-Shyang Jeng,** Departments of Surgery, Mackay Memorial Hospital, Taipei, Taiwan, China
**Shou-Chuan Shih, Po-Chuan Wang, Wen-Hsiung Chang, Horng-Yuan Wang, Li-Rung Shyung, Shee-Chan Lin, Chin-Roa Kao,** Department of Internal Medicine, Mackay Memorial Hospital, Taipei, Taiwan, China
**Yi-Chun Tsai, Tsu-Yen Wu,** Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, China
**Kuo-Shyang Jeng,** Mackay Junior School of Nursing, Taipei,Taiwan, China
**Supported by** the grants from the Department of Health, National Science Council, Executive Yuan, Taiwan (Dr. Jeng) (NSC 86-2314-B-95-001)
**Correspondence to:** Kuo-Shyang Jeng, M.D., F.A.C.S.Department of Surgery, Mackay Memorial Hospital, No. 92, Sec 2, Chung-San North Road, Taipei, Taiwan, China. issheen.jks@msa.hinet.net
**Telephone:** +886-2-25433535 **Fax:** +886-2-27065704
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## Abstract

**AIM:** This study was to investigate whether surgery could increase cancer dissemination and postoperative recurrence in patients with hepatocellular carcinoma (HCC) by detection of human α-fetoprotein messenger RNA (hAFP mRNA). hAFP mRNA in the peripheral blood of patients with HCC has been considered as a surrogate marker for circulating tumor cells.

**METHODS:** Eighty-one consecutive patients who underwent curative resection for HCC entered this prospective cohort study. We examined hAFP mRNA from the peripheral blood obtained preoperatively, perioperatively, and postoperatively to correlate the prognosis after curative resections from HCC patients and from the control subjects. Detection of hAFP mRNA by reverse transcriptase and polymerase chain reaction amplification (RT-PCR) was performed with primers specifically. The relations between the clinical variables (age, sex, associated liver cirrhosis, hepatitis B virus infection, hepatitis C virus infection, serum α-fetoprotein and Child-Pugh class), the histological variables (size, capsule, vascular permeation, grade of differentiation, and daughter nodules), hAFP mRNA in peripheral blood of 3 different sessions, and postoperative course (recurrence, and recurrence related death) were analysed.

**RESULTS:** No hAFP mRNA was detected in control group subjects. Twenty-two (27%), 24 (30%) and 19 (23%) of 81 HCC patients had hAFP mRNA positivity in the preoperative, perioperative and postoperative peripheral blood. The preoperative presence did not influence the risk of HCC recurrence (55% *vs* 41%, $P=0.280$). In contrast, patients with postoperative presence had a significantly higher recurrence (90% *vs* 31%, $P<0.001$; odds ratio 19.2; 95% confidence interval: 4.0-91.7). In the multivariate analysis by COX proportional hazards model, postoperative positivity had a significant influence on recurrence ($P=0.067$) and recurrence related mortality ($P=0.017$). Whereas, the perioperative positivity of hAFP mRNA did not increase HCC recurrence (58% *vs*.39%, $P=0.093$). The correlation between perioperative hAFP mRNA positivity and recurrence related mortality had no statistical significance ($P=0.836$).

**CONCLUSION:** From our study, perioperative detection of hAFP mRNA in peripheral blood of patients has no clinical relevance and significant role in the prediction of HCC recurrence. Surgical resection itself may not accelerate cancer dissemination and does not increase postoperative recurrence significantly either.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is the leading malignancy with a poor prognosis in areas of high hepatitis B and C prevalence[1-4]. After curative resections of HCC, a lage proportion of patients develop tumor recurrence within the first 3 years. Whether intraoperative manipulation contributes to cancer dissemination remains a debating subject.

How to detect these disseminated cancer cells in the perioperative period is a problem. The isolation and identification of tumor cells in a small blood sample by conventional methods is very difficult because the number of malignant cells in the circulation may be extremely small[5-8]. Recently, it has become possible and sensitive to identify tumor-specific gene transcripts (messenger RNA/circular DNA) by means of polymerase chain reaction (PCR). With the use of PCR-based method which permits detection of 1 tumor cell among $10^7$ normal peripheral, mononuclear blood cells, the blood-borne dispersion of tumor cells during surgical manipulation has been reported in humans with prostatic carcinoma, melanoma, breast carcinoma and pancreatic carcinoma[9-12].

For detecting HCC cells in circulation, reverse transcriptase (RT) PCR targeting human alphafetoprotein (hAFP) messenger RNA (mRNA) or human albumin mRNA has been proposed[13-22]. Lack of the specificity of albumin mRNA has been emphasized[21,22]. Human AFP mRNA has been accepted as a liver specific and cancer-specific marker[23,24]. Many clinical studies have suggested that hAFP mRNA in peripheral blood can be used as a surrogate marker of circulating HCC cells and as a prognostic indicator in patients treated with ethanol injection and/or arterial embolization[18].

In an attempt to elucidate whether surgical resection of HCC accelerated cancer dissemination, we designed this prospective study.

## MATERIALS AND METHODS

### Study population

From August 1995 to July 1999, 81 consecutive patients [42 men and 39 women, with a mean age of 52±13 years (range: 16 to 79 years)] with HCC undergoing curative hepatic resection at Mackay Memorial Hospital, Taipei,Taiwan, China, were enrolled in this prospective study (Table 1). Patients who had previously or simultaneously other malignant disorders, and who had previously received hepatectomy, intraoperative blood transfusion, preoperative and postoperative hepatic arterial chemoembolization (TACE) or neoadjuvant ethanol injection were all excluded.

**Table 1** Demographic data including clinical and tumor variables of patients with HCC undergoing curative resection ($n$=81)

| Variables | No. of patients (%) |
|---|---|
| Age (mean, years) | 52±13 |
| Male | 42 (52) |
| Cirrhosis | 56 (69) |
| Child- Pugh' s class A | 70 (86) |
| HBsAg (+) | 62 (77) |
| Anti-HCV (+) | 31 (38) |
| Serum AFP <20 ng/ml | 29 (36) |
| 20-$10^3$ ng/ml | 29 (36) |
| >$10^3$ ng/ml | 23 (28) |
| Size of HCC < 3 cm | 25 (30) |
| 3-10 cm | 28 (35) |
| > 10 cm | 28 (35) |
| Complete capsule | 28 (35) |
| Daughter nodules | 32 (40) |
| Vascular permeation | 37 (46) |
| Edmondson-Steiner' s grade I | 6 (7) |
| grade II | 36 (44) |
| grade III | 26 (32) |
| grade IV | 13 (16) |

AFP: serum alpha fetoprotein, HBsAg(+): positive hepatitis B surface antigen, Anti-HCV(+): positive hepatitis C virus antibody, Edmondson and Steiner grade: differentiation grades I, II, III, and IV.

The hepatectomy procedure was selected according to the patient' s liver function and cancer location. Prior to resection, intraoperative ultrasonography to scan the entire hepatic field was performed in every patient. Surgery was defined as curative when all gross lesions were removed with an over 1 cm free resection margin which was proven tumor-free histologically. The surgical procedures included 62 major resections (6 extended right lobectomies, 18 right lobectomies, 14 left lobectomies, and 24 double segmentectomies) and 19 minor resections (11 segmentectomies, 5 subsegmentectomies, and 3 wedge resections).

Peripheral blood samples for detection of hAFP mRNA were obtained from all study patients from forearm one day prior to surgery (preoperation), immediately (i.e., within 12 hours) after liver resection (perioperation), and 90 days after surgery (postoperation) from all 81 patients.

After discharge, all the patients were followed up at our outpatient clinic and received regular clinical assessments to detect tumor recurrence including periodic abdominal ultrasonography (every 2-3 months during the first 5 years, then every 4-6 months thereafter), and serum AFP and liver biochemistry (every 2 months during the first 2 years, then every 4 months during the following 3 years, and every 6 months thereafter). Abdominal computed tomography scans (CT) were also done (every 6 months during the first 5 years, then every year). Hepatic arteriography was obtained if there was a suspicion of cancer recurrence from ultrasonography or CT scan, or serum AFP. Chest X ray to detect pulmonary metastasis was done every 6 months. Bone scan to detect osseous metastasis was undertaken every 6 months. Detection of tumor on any imagimg studies was defined as "clinical recurrence".

Parameters analyzed for recurrence included sex (male *vs*. female), age, the presence of liver cirrhosis, Child-Pugh class of liver functional reserve (A *vs*. B), hepatitis B virus infection (hepatitis B surface antigen), hepatitis C virus infection (anti-hepatitis C virus antibody), serum AFP level (<20 ng/ml *vs*. 20-1 000 ng/ml *vs*. >1 000 ng/ml), tumor size (<3 cm *vs*. 3-10 cm *vs*. >10 cm), tumor encapsulation (complete *vs*. incomplete or absent), presence of daughter nodules, and vascular permeation (including vascular invasion and/or tumor thrombi, in either the portal vein or hepatic vein), and cell differentiation (Edmondson and Steiner grades I, II, III and IV)(Table1).

A control group included 30 healthy volunteers without liver disease and 20 patients with chronic liver disease without evidence of HCC. They also received hAFP mRNA detection from peripheral blood.

### Detection of hAFP mRNA

We used human hepatocytes to determine the sensitivity of the assay. Using tetradecytrimethyl- ammonium bromide, nucleated cells were isolated from peripheral blood. Total RNA was extracted from cryopreserved liver tissues. The sequences of the sense primers were 5' -ACT GAA TCC AGA ACA CTG CAT AG-3' (external-sense) and 5' -TGC AGT CAA TGC ATC TTT CAC CA-3' (internal-sense) and those of the antisense primers 5' -TGG AAT AGC TTC CAT ATT GGA TTC- 3' (external-antisense) and 5' -AAG TGG CTT CTT GAA CAA ACT GG- 3' (internal-antisense). The sizes of the amplified products of hAFP mRNA were 176 and 101 base pairs by external and internal primer pairs, respectively.

A Hep G2 (hepatoblastoma) cell line served as positive control for hAFP mRNA expression. For negative controls, we used EDTA treated water (filtered and vaporized). With cDNA derived from Hep G2, specific bands for hAFP (101 bp) were observed. In contrast, the cell lines that served as negative controls did not yield these bands. It was also impossible to detect free RNA extracted from 5 ml aliquots of control blood, in which Hep G2 cells were suspended. The sensitivity of our assay, determined in a dilution experiment using freshly isolated human hepatocytes ($10^5$ to $10^1$) in 1 ml whole blood before RNA extraction, was approximately 1 hepatocyte for every $10^5$ peripheral mononuclear cells.

Ethylenediamine tetraacetic acid (EDTA)-treated whole blood was centrifuged and the plasma fraction was removed. The cellular fraction was enriched for mononuclear cells (MNC) or possible tumor cells according to the method described by Oppenheim. Total cellular RNA was then extracted with PUREscript RNA isolation kits TRI-Zol (Life Technologies Inc., Gaithersburg, USA), from 5 μg of which cDNA was synthesized. The reverse transcription reaction solution contained 6 μl of 5×first strand buffer, 10 mM dithiothreitol, 125 mM each of dCTP, dATP, dGTP and dTTP, 0.3 μg of random hexamers, and 200 units of superscriptase II moloney murine leukemia virus reverse transcriptase (Life Technologies Inc.). The RNA solution was incubated at 95 ℃ for 10 minutes, quickly chilled on ice, then mixed with the reverse transcription reaction solution (total volume 20 μl), and incubated at 37 ℃ for 60 minutes. The first PCR reaction solution contained 5 μl of the synthesized cDNA solution, 10 μl of 10X polymerase reaction buffer, 500 μM each of dCTP, dATP, dGTP and dTTP, 15 pmol of each external primer (EX-sense and EX-antisense), 4 units of Thermus Brockiamus Prozyme DNA polymerase (PROtech Technology Ent. Co., Ltd. Taipei, Taiwan, China), and water. The PCR cycles were: denaturing at 94 ℃ for 1 minute, annealing at 52 ℃ for 1 minute,

and primer extension at 72 ℃ for 1 minute. The cycles were repeated 40 times. The PCR product was reamplified with internal primers for nested PCR to obtain a higher sensitivity. The first and second PCR components were the same, but for the primer pairs (IN-sense and IN-antisense), the final product was electrophoresed on 2 % agarose gel and stained with ethidium bromide for the specific band of 101 base pairs.

### Statistical analysis

A statistical software (SPSS for Windows, version 8.0, Chicago, Illinois) was used, with Student' s *t*-test for continuous variables, $\chi^2$ test or Fisher' s exact test for categorical variables. Stepwised logistic regression and COX proportional hazards model were used for multivariate stepwise analysis to identify independently significant factors in predicting recurrence and mortality. A *P* value <0.05 was defined as significant.

## RESULTS

### No hAFP mRNA was detected in peripheral blood of all patients in control group

In patients with HCC, hAFP mRNA in peripheral blood was detected in 27% (22/81), 30% (24/81), and 23% (19/81) preoperatively, perioperatively and postoperatively, respectively. According to hAFP mRNA status, we classified patients into 8 groups. For example, in group 1, hAFP mRNA was consistently positive preoperatively, perioperatively,and postoperatively; in group 2, positive preoperatively and perioperatively but negative postoperatively; and in group 8, consistently negative preoperatively, perioperatively, and postoperatively, *etc* (Table 2).

Thirty-six patients (44.4%) had clinically detectable recurrence during the follow-up period (median 3 years, range 2-5 years), of whom 25 died. The presence of hAFP mRNA preoperatively did not correlate with the risk of recurrence (55% *vs*. 41%, *P*=0.280) and recurrence related mortality (*P*=0.7283) (Tables 3 and 4). In contrast, patients with postoperative hAFP mRNA had a significantly higher recurrence rate (90% *vs*. 31%, *P*<0.001), with an odds ratio of 19.2 (95% confidence interval [CI]: 4.0-91.7), which was also significantly associated with recurrence related mortality (*P*=0.017) (in the multivariate analysis by COX proportional hazards model) (Tables 3 and 4). The presence of hAFP mRNA perioperatively did not significantly correlate with the risk of recurrence (58% *vs*. 39%, *P*=0.093) and the recurrence related mortality (*P*=0.836) (Tables 3 and 4).

On multivariate analysis, the significant predictors of recurrence included vascular premeation (*P*=0.023), grade of cellular differentiation (*P*=0.007) and postoperative hAFP mRNA positivity (*P*<0.001 by univariate; *P*=0.067 by multivariate, weak significance) (Table 3). The significant parameters correlating with mortality after recurrence consisted of grade of cellular differentiation (*P*=0.057, weak significance) and postoperative hAFP mRNA (*P*=0.017) (Table 4).

**Table 3** Predictors of HCC recurrence

| Variables | *P* values | |
|---|---|---|
| | UV | MV |
| Sex | 0.274 | - |
| Age | 0.842 | - |
| Liver cirrhosis | 0.019 | - |
| HBsAg (+) | 0.505 | - |
| Anti-HCV (+) | 0.622 | - |
| Serum AFP | <0.001 | - |
| Child-Pugh class | 0.087 | - |
| Size (<3 cm, >10 cm) | 0.140 | - |
| Capsule | <0.001 | n.s. |
| Daughter nodules | <0.001 | n.s. |
| Vascular permeation | <0.001 | 0.023 |
| Edmondson Steiner grade | <0.001 | 0.007 |
| Preoperative hAFP mRNA(+) | 0.280 | - |
| Preioperative hAFP mRNA(+) | 0.093 | - |
| Postoperative hAFP mRNA(+) | <0.001 | 0.067 |

UV: univariate analysis, MV: multivariate analysis, AFP: serum alpha fetoprotein, HBsAg(+): positive hepatitis B surface antigen, Anti-HCV(+): positive hepatitis C virus antibody, Edmondson and Steiner grade: differentiation grades I, II *vs*. III, IV, n.s.: not significant.

**Table 4** Correlation between variables and recurrence related mortality

| Variables | *P* values | |
|---|---|---|
| | UV | MV |
| Sex | 0.815 | - |
| Age | 0.930 | - |
| Liver cirrhosis | 0.039 | n.s. |
| HBsAg (+) | 0.835 | - |
| Anti-HCV (+) | 0.548 | - |
| Serum AFP | <0.000 | - |
| Child-Pugh class | 0.092 | - |
| Size (<3 cm, >10 cm) | 0.274 | - |
| Capsule | 0.004 | n.s. |
| Daughter nodules | 0.004 | n.s. |
| Vascular permeation | <0.001 | n.s. |
| Edmondson Steiner grade | <0.001 | 0.057 |
| Preoperative hAFP mRNA (+) | 0.728 | - |
| Perioperative hAFP mRNA (+) | 0.835 | - |
| Postoperative hAFP mRNA (+) | <0.001 | 0.017 |

UV: univariate analysis, MV: multivariate analysis, AFP: serum alpha fetoprotein, HBsAg (+): positive hepatitis B surface antigen, Anti-HCV(+): positive hepatitis C virus antibody, Edmondson Steiner grade: differentiation grades I, II *vs* III, IV, n.s. :not significant.

**Table 2** Correlation among timing of blood sample collection and circulating tumor cell status and postoperative recurrence

| Group | Preoperation (baseline) | Perioperation (within 12 hours after surgery) | Postoperation (90 days after surgery) | Number of patients | Patient number of recurrence (%) |
|---|---|---|---|---|---|
| 1. | Positive | Positive | Positive | 9 | 8 (88.9) |
| 2. | Positive | Positive | Negative | 3 | 2 (66.7) |
| 3. | Positive | Negative | Positive | 2 | 2 (100) |
| 4. | Positive | Negative | Negative | 8 | 0 (0) |
| 5. | Negative | Positive | Positive | 5 | 4 (80) |
| 6. | Negative | Positive | Negative | 7 | 0 (0) |
| 7. | Negative | Negative | Positive | 3 | 3 (100) |
| 8. | Negative | Negative | Negative | 44 | 17 (38.6) |
| Overall | | | | 81 | 36 (44.4) |

## DISCUSSION

Surgical dissemination of tumor cells has been observed in various solid cancers and manipulation *per se* has been regarded as the main cause. According to Nishizaki, using inoculation of VX2 carcinoma into rabbit liver, manual manipulation of a tumor might well enhance metastasis[25]. According to Liotta, tumor massage resulted in at least a 10-fold rise over the initial concentration of tumor cells, as well as a higher proportion of large clumps[26]. Yamanaka *et al*. demonstrated a large quantity of HCC cells in the portal vein during hepatic resection[27]. In our series, 30% of patients had the presence of hAFP mRNA perioperatively. The detection rate seemed higher than that of preoperation (27%), and postoperation (23%), but the difference had no statistical significance among them. In addition, this increase did not correlate with postresection recurrence. Further more, from individual group point of view, we found the alteration from preoperative negativity to perioperative positivity in groups 5 and 6 (Table 2). Whereas the postoperative recurrence varied greatly (80% *vs* 0%) between them. Statistically, perioperative detection did not contribute to cancer dissemination (Table 3).

The interpretation of detection of hAFP mRNA remains controversial. From our study, among 3 different sessions of blood sampling, only postoperative detection of hAFP mRNA correlated significantly with postresection recurrence related mortality. We proposed that some possible factors contributed to the different significance among the three different blood sampling times.

The detection before surgery may be attributed to the cells released spontaneously from primary tumor *in situ*. HCC tissue is surrounded by a vascular space analogous to the hepatic sinusoids. Because of this anatomic structure, tumor cells might easily be released into the sinusoids spontaneously. Thereafter, they might migrate into the portal or hepatic vein and finally enter the systemic circulation. However, from our study, these preoperative circulating HCC cells had no prognostic significance.

Molecular methods now permit us to detect a small number of cancer cells in the blood by use of RT-PCR targeting a cell-specific gene. Studies in animal models have indicated that at least $10^4$ circulating tumor cells are required for metastasis to develop. To date, however, the absolute number of cells required for metastasis in the human circulation is unclear and even if cancer cells are detected in the circulation, their potential to develop metastatic foci is unknown. When malignant cells were released into the circulation, a variety of host and tumor cell factors could determine their distribution and fate[28-33]. Most circulating HCC cells may rapidly die in the blood by various host immune and non-immune defenses, and are destroyed by mechanical forces, including turbulence and the trauma associated with vascular adhesion and transcapillary passage, or lysed by lymphocytes, monocytes, and natural killer (NK) cells. Some tumor cells are nonspecifically trapped or specifically arrested in the first capillary bed encountered. Circulating tumor cells trapped in a given location could then recirculate and arrest at other locations, then grow into tumor colonies[29-33].

Okuda *et al*[34] and Komeda *et al*[14] could not detect any hAFP gene transcripts in patients with liver metastases or in healthy persons. Ijichi *et al* considered this RT-PCR assay targeting hAFP mRNA as a sensitive and specific method for detecting HCC cells in the circulation *in vivo* and *in vitro* experiments, and no positivity was found in any healthy controls[35]. From our study, hAFP mRNA could not be detected from all the 50 controls. However, some authors suggested that AFP gene wass not hepatoma-specific, but rather a liver-specific marker[13,15,17,18,36,37].

Surgery itself may increase the release of liver cells, not only HCC cells but also normal hepatocytes. Both kinds of cells may contribute to the positivity of hAFP mRNA. According to Louha M, not only liver surgery but also nonsurgical invasive managements such as needle liver biopsy or intervention therapies such as TACE, chemotherapy and ethanol ablation therapy, the increased shedding of either HCC cells or normal hepatocytes into circulation might contribute to the increase of detection rate of hAFP mRNA[38]. This was also the reason why we excluded those who had received these intervention therapies from the current study.

It has become a fact that RT-PCR based tests lose its specificity for HCC cell detection when they are performed on samples obtained immediately after surgical or nonsurgical invasive procedures. This pitfall may also account for the gap between the frequency of cell detection after surgery and the expected tumor recurrence rate. A consensus existed that hAFP mRNA might not be regarded as specific markers of HCC cells if blood samples were taken during liver surgery[39].

Secondly, the different site of blood sampling might contribute to the discrepancy. Central venous blood in Kienle' s study was drawn before it passed through any capillary bed, with only a short distance after leaving the liver, the cells expressing hAFP might not undergo apoptosis or were not filtered out in capillary beds, therefore possibly accounting for the high detection rate (46%) during surgery[37]. Another factor influencing the detection of hAFP mRNA in intraoperative central venous blood samples might be by a "dilution effect" following intraoperative blood transfusion[37]. This was the reason why we excluded those receiving transfusion from our study.

Thirdly, the different blood sampling time might contribute to the discrepancy. Similarly, from the literature, in intraoperative detection of other tumors, this factor also existed. Brown *et al* sampled blood at the time of maximum tumor manipulation and postoperative 24 hours in those with breast cancer[11]. Eschwege P obtained blood samples 5 minutes after prostate carcinoma removal[9]. Warr RP obtained blood samples at 2 different sessions and 24 hours postoperatively in those with malignant melanoma[10]. According to Hayashi N, the blood samples were obtained through a catheter in the portal vein before, during, and after manipulation of colorectal cancer[40] .

Lemonie obtained peripheral blood samples at two different intervals: the first, during the exploratory phase and the second, after hepatectomy was completed[41]. Witzigmarn obtained blood samples before and during the operation (after mobilization of the liver), and on the second postoperative day[39]. Louha obtained peripheral blood samples before treatment, 1 hour and 24 hours after percutaneous ethanol injection or TACE treatment[38]. Witzigmann obtained blood samples on the second day after TACE[39].

Louha found unexpectedly that liver cells began spreading at an early stage during surgery, i.e., after liver mobilization and rotation, before liver parenchyma transection. This was probably related to the sponge-like structure of the liver and to the stretching and compression of the organ during liver mobilization[42]. Surgery-related liver cell spreading also occurred more frequently, compared with that induced by needle liver biopsy. This difference of cell number was probably related to the different degree of manipulation on the liver between resection and biopsy.

In the present study, we selected the sampling time within the first 12 hours after hepatectomy because of two reasons. First, the so-called "maximal manipulation" during surgery was usually difficult to define. The degrees of manipulation among the mobilization of the liver, or the division of important vessels and ducts of the segment or lobe, and the dissection of

hepatic parenchyma, were difficult to quantitate. In addition, the detailed procedure among individual patients varied. Second, we believed that within 12 hours after resection, the released cells, if present, might still remain. Funaki, Okuda, and Ijichi thought destruction of circulating HCC cells transiently liberated during surgery needed 7 days[15,34,35].

From prognostic point of view, in literature, whether the shedding of cancer cells during intraoperative manipulation contributed to cancer dissemination and postoperative recurrence has remained debatabe[33].

Witzigmann[39] and Lemonie[41] did not find any correlation between postresection recurrence of HCC and the presence of hAFP mRNA irrespective of whether it was measured before, during, or after surgery. Lemonie mentioned that his result concurred with other experimental and clinical data, suggesting that release of abnormal cells in the circulation, either spontaneously or secondary to surgical manipulation, was an intermittent and transient phenomenon[41]. Okuda found that most patients whose hAFP mRNA was not detected in peripheral blood perioperatively were diagnosed as free of intrahepatic recurrence or distant metastasis within 9 months after the operation[34].

In contrast, Ijichi *et al*. suggested that surgical dissemination might actually cause HCC recurrence within a short period[35]. The fact that alteration from negative to positive hAFP mRNA throughout the perioperative time might indicate a high risk of recurrence has been emphasized by Okuda[34]. Funaki *et al* reported that hAFP mRNA positive 2-3 days after the operation might be thought of as a high risk indicator of recurrence[15]. Ferris found HCC recurred in 28% of patients with HCC after orthotopic liver transplantation[43]. Ferris inferred that circulating HCC cells were present in the peripheral blood even after removal of the diseased liver, and that these residual tumor cells formed intra-and extra-hepatic metastatic foci after transplantation. To decrease cancer dissemination, we suggest that forceful mobilization or manipulation of the liver has to be avoided.

In addition, some studies have proposed anesthesia and unrelated surgery promote the spread of malignant disease[44]. It is another challenging issue whether surgery per se, or general anesthesia *per se*, or both, may change the immune system of the host perioperatively and increase the opportunity of postoperative cancer spread.

Based on Salo' s animal studies, during an operation, operative trauma was generally considered to have a greater role than anaesthesia in altering immune responses. The immune responses to major surgery, and operative complications resulting in massive mediator release might place the patient at risk[44].

Recent investigations have suggested that general anesthesia may cause an unregulated activation of the process of apoptosis leading to lymphocytopenia and immune suppression resulting in different response in B-lymphocytes (but not in T-lymphocytes), natural killer cell activity or antibody-dependent cellular cytotoxicity 3-4 days after surgery[44,45]. The true trigger mechanisms are still unclear. However, lymphocytopenia was not found in our patients, and it might not have significant contribution to recurrence. The association between transfusion-induced immunosuppression and poorer prognosis in patients with cancer has also been mentioned[46]. It was also the reason why we excluded those receiving intraoperative transfusion from this study.

The postoperative presence of circulating HCC cells may therefore represent surviving malignancy that can continue the metastatic process. The possible explanations are as follows. A proportion of cancer cells released from the resected tumor (s) survive in the circulation for a long time without being destroyed, or the presence of unresected occult metastases are undetectable at the time of surgery, or a newly developing malignant focus is too small to be detected by conventional follow-up studies.

Perioperative detection of hAFP mRNA has no relevant and significant role in the prediction of prognosis. We suggest surgical resection itself accelerate cancer dissemination and does not increase postoperative recurrence significantly either.

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