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Usefulness of Tumor Tissue Biopsy for Predicting the Biological Behavior of Hepatocellular Carcinoma

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Abstract. *Background/Aim: Assessment of the biological behavior of tumors is important for choosing an appropriate cancer therapy. In hepatocellular carcinoma (HCC), the biological behaviour can be assessed by tumor morphology and molecular biology. This study investigated the usefulness of tumor tissue biopsy for predicting the biological behavior of HCC. Patients and Methods: We studied 43 patients who underwent hepatectomy and preoperative liver tumor biopsy for HCC. We performed clinicopathological and immunohistochemical (IHC) analyses. The expression of the following molecules was examined: regulator of G-protein signaling 5 (RGS5), glypican-3 (GPC3), keratin 19 (K19), epithelial cell adhesion molecule (EpCAM), protein induced by vitamin K absence or antagonist-II (PIVKA-II), β-Catenin, and p53. Results: There was an overall 83.7% agreement regarding tumor differentiation between the preoperative biopsy specimens and the resected specimens. The accuracy of IHC analysis was more than 70% for all molecules between the preoperative biopsy specimens and the resected specimens. The RGS5-positive biopsy cases had higher serum α-fetoprotein levels (p=0.04), a higher rate of moderately or poorly differentiated tumors (p=0.02) and portal vein invasion (p=0.0003) than the RGS5-negative biopsy cases. The GPC3-positive biopsy cases were younger (p=0.04), had higher serum PIVKA-II levels (p=0.01), and a higher rate of portal vein invasion (p=0.03) than the GPC3-negative biopsy cases. The PIVKA-II-positive biopsy cases had significantly higher serum PIVKA-II levels than the PIVKA-II-negative biopsy cases (p=0.02). The other molecular markers showed no significantly different clinical findings between the positive and negative cases. Conclusion: In HCC, there was a high agreement rate of both the histopathological and IHC findings between preoperative biopsy specimens and resected specimens. In the biopsy specimens of HCC, RGS5 and GPC3 expression were useful molecular makers for predicting portal vein invasion. Liver tumor biopsy is useful for predicting the biological behavior of HCC through histopathological and immunohistochemical findings.*

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Worldwide, liver cancer is the fourth most common cause of cancer-related death and ranks sixth in terms of incident cases (1). Primary liver cancer includes hepatocellular carcinoma (HCC) and cholangiocellular carcinoma. HCC accounts for most primary liver cancers.

Individualized therapy is needed for patients with cancer including those with HCC. In HCC, studies based on tumor histopathological and molecular biology are ongoing. Recently, treatments based on molecular classifications of HCC and tumor phenotypes of HCC associated with genetic mutations or molecular classifications have been reported (2, 3). In addition, immunohistochemical studies indicated that the expression of keratin 19 (K19) and epithelial cell adhesion molecule (EpCAM) correlates with the prognosis of HCC patients (4). However, most of these studies analyzed resected HCC specimens, thus the usefulness of tumor tissue biopsy for predicting the biological behavior of HCC is yet unclear.

This study investigated the usefulness of tumor tissue biopsy for predicting the biological behavior of HCC. We compared pairs of preoperative liver tumor biopsies and surgically resected specimens on histopathological findings and immunohistochemical findings.

Patients and Methods

Materials. This retrospective study of human specimens enrolled 43 cases of HCC (28 men and 15 women, median age=67±9 years) who underwent hepatectomy with preoperative percutaneous transhepatic liver tumor biopsy at the Kurume University Hospital in Japan from 2007 to 2013. Histopathological and immunohistochemical (IHC) analyses were conducted in all preoperative liver tumor biopsies and surgically resected specimens. This study was approved by the Ethical Committee at Kurume University, approval ID number: 18120. The Ethical Committee waived the requirement for written informed consent for cases because the data for these patients were retrospectively analyzed.

Histopathological analysis. Each tissue was fixed with 10% formalin, embedded in paraffin, cut into 5-μm sections, and then used for histological analyses. The specimens were stained with hematoxylin–eosin and examined under a light microscope. Histopathological findings were assessed according to World Health Organization classification guidelines, 5th edition (5). Differentiation of HCC tumors was classified into well, moderately, and poorly differentiated types. Histological growth pattern assessments were based on the tumor cell arrangements, which comprised ≥50% of the overall histological growth patterns and were categorized as thin trabecular type (thin), thick trabecular type (thick), pseudoglandular type (ps), macro trabecular-massive type (macro), or solid type (solid) (6). Representative histological growth patterns of HCC in resected specimens and in biopsy specimens are shown in Figure 1.

IHC analysis. Immunostaining was performed on paraffin-embedded sections. The following primary antibodies were used: regulator of G-protein signaling 5 (RGS5) (clone 1C1, 1:250 dilution, Novus Biologicals, LLC, Littleton, CO, USA), glypican-3 (GPC3) (clone 1G12, 1:1 dilution, Nichirei Bioscience Inc., Tokyo, Japan), K19 (clone RCK108, 1:25 dilution, Dako; Agilent Technologies, Inc., Santa Clara, CA, USA), EpCAM (clone VU1D9, 1:50 dilution, Cell Signaling Technology, Danvers, MA, USA), Protein induced by vitamin K absence-II (PIVKA-II) (clone MU-3, 1:500 dilution, EIDIA, Tokyo, Japan), β-Catenin (clone β-Catenin-1, 1:200 dilution, Dako; Agilent Technologies, Inc., Santa Clara, CA, USA), and p53 (clone Pab1801, 1:200 dilution, Thermo Fisher Scientific, Waltham, MA, USA).

RGS5 immunostaining was performed using the CSAII system (Dako, Agilent Technologies, Inc. Santa Clara, CA, USA) following the manufacturers' protocol and based on a previous report (7). Immunostaining of GPC3, K19, EpCAM, PIVKA-II, β-Catenin, and p53 was performed using the BenchMark ULTRA automatic immunostainer (Ventana Automated Systems, Inc. Tucson, AZ, USA). The positive expression of tumor cells in biopsy specimens taken from HCC is shown in Figure 2. When 5% and more tumor cells showed a positive reaction, we determined the specimen as positive. Histopathological and immunohistochemical findings were assessed by two pathologists (TS and RK). The pathologists were blinded to the patient information, and the findings were assessed independently.

Statistics. All data are expressed as the mean±SD, unless otherwise indicated. Pearson's Chi-square test was used to compare nominal and ordinal variables between the two groups. Analysis of variance and *t*-tests were used to compare continuous variables between the groups. A p-value <0.05 was considered statistically significant. Statistical analysis was conducted using JMP Pro version 13 software (SAS Institute Japan Ltd., Tokyo, Japan).

Results

Clinicopathological findings. The clinicopathological findings are summarized in Table I. Among the resected specimens the ratio of well, moderately, and poorly differentiated tumors was 6:35:2. There was an overall 83.7% agreement regarding diagnoses of tumor differentiation between pairs of preoperative biopsies and resected specimens. Among the resected specimens, the histological growth patterns of six cases were determined to be the macro trabecular type or the solid type. There was an overall 74.4% agreement on histological growth patterns between pairs of preoperative biopsies and resected specimens.

Immunohistochemical findings of preoperative biopsies and resected specimens. The immunohistochemical findings are shown in Figure 3 and summarized in Table II. The immunohistochemical accuracy between pairs of preoperative biopsies and resected specimens was 70% for RGS5, 86% for GPC3, 98% for EpCAM, 88% for PIVKA-II, 81% for β-Catenin, and 72% for p53. K19 expression was negative in all biopsy specimens. Staining for K19 located in the cell membrane and cytoplasm was judged as positive, and one case was positive among the resected cases.

Clinicopathological features and immunohistochemical findings. The clinicopathological features and immunohistochemical findings are shown in Tables III to VIII. The RGS5-positive biopsy cases had higher serum α-fetoprotein (AFP) levels (p=0.04), and had a higher rate of moderately or poorly differentiated tumors (p=0.02), the macro trabecular type or the solid type (p=0.003), and portal vein invasion (p=0.0003) than the RGS5-negative biopsy cases. In resected specimens, portal vein invasion was observed in 16 of 19 RGS5-positive biopsy cases. The GPC3-positive biopsy cases were found to be younger (p=0.04), had higher serum PIVKA-II levels (p=0.01) and had a higher rate of the macro trabecular type or solid type (p=0.001) and portal vein invasion (p=0.03) than the GPC3-negative biopsy cases. In resected specimens, portal vein invasion was observed in seven of eight GPC3-positive biopsy cases. The PIVKA-II-positive biopsy cases had significantly higher serum PIVKA-II levels than the PIVKA-II-negative biopsy cases (p=0.02). None and three biopsy cases were positive for K19 and EpCAM, respectively. β-Catenin expression showed no significant differences between the positive and negative cases. p53 expression was significantly associated with increased macro or solid type (p=0.03).

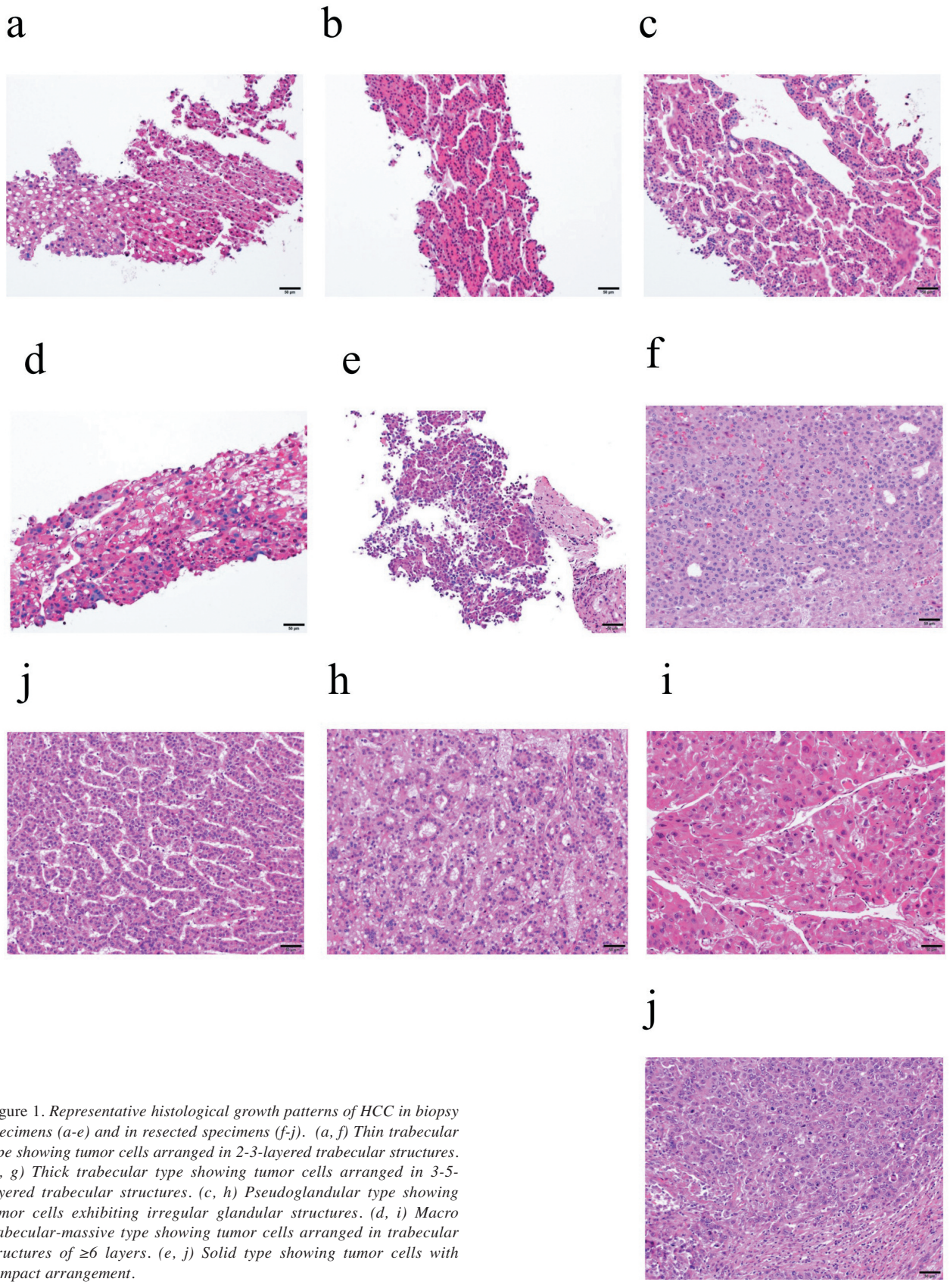

Figure 1. *Representative histological growth patterns of HCC in biopsy specimens (a-e) and in resected specimens (f-j). (a, f) Thin trabecular type showing tumor cells arranged in 2-3-layered trabecular structures. (b, g) Thick trabecular type showing tumor cells arranged in 3-5-layered trabecular structures. (c, h) Pseudoglandular type showing tumor cells exhibiting irregular glandular structures. (d, i) Macro trabecular-massive type showing tumor cells arranged in trabecular structures of ≥6 layers. (e, j) Solid type showing tumor cells with compact arrangement.*

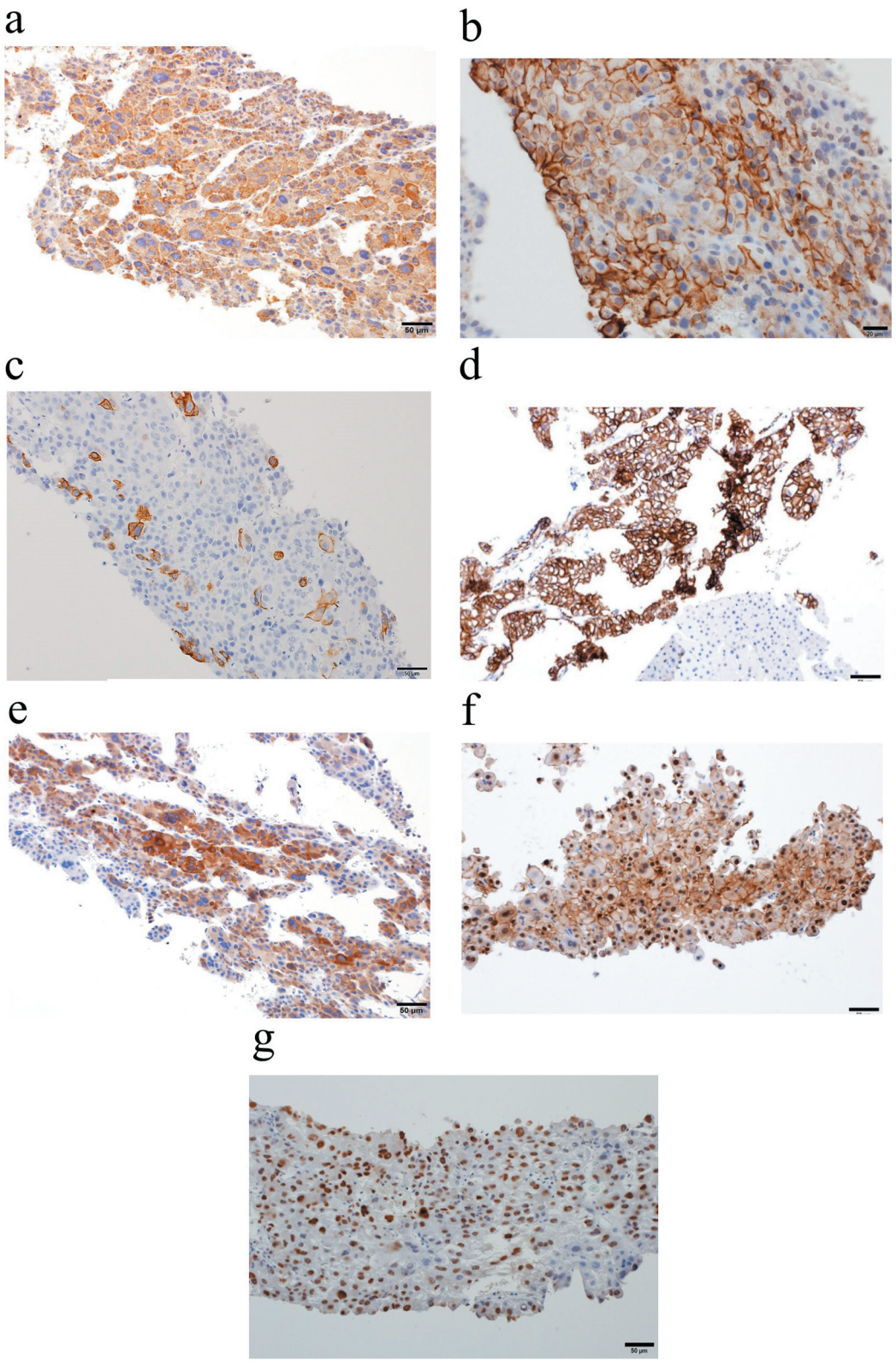

Figure 2. *Representative immunohistochemical findings of RGS5 (a), GPC3 (b), K19 (c), EpCAM (d), PIVKA-II (e), β-Catenin (f), p53 (g) in biopsy HCC specimens. Positive immunostaining for RGS5 or PIVKA-II is noted in the cytoplasm, that for GPC3 in the cell membrane, that for K19 or EpCAM in the cell membrane and cytoplasm, and that for β-Catenin or p53 in the nucleus.*

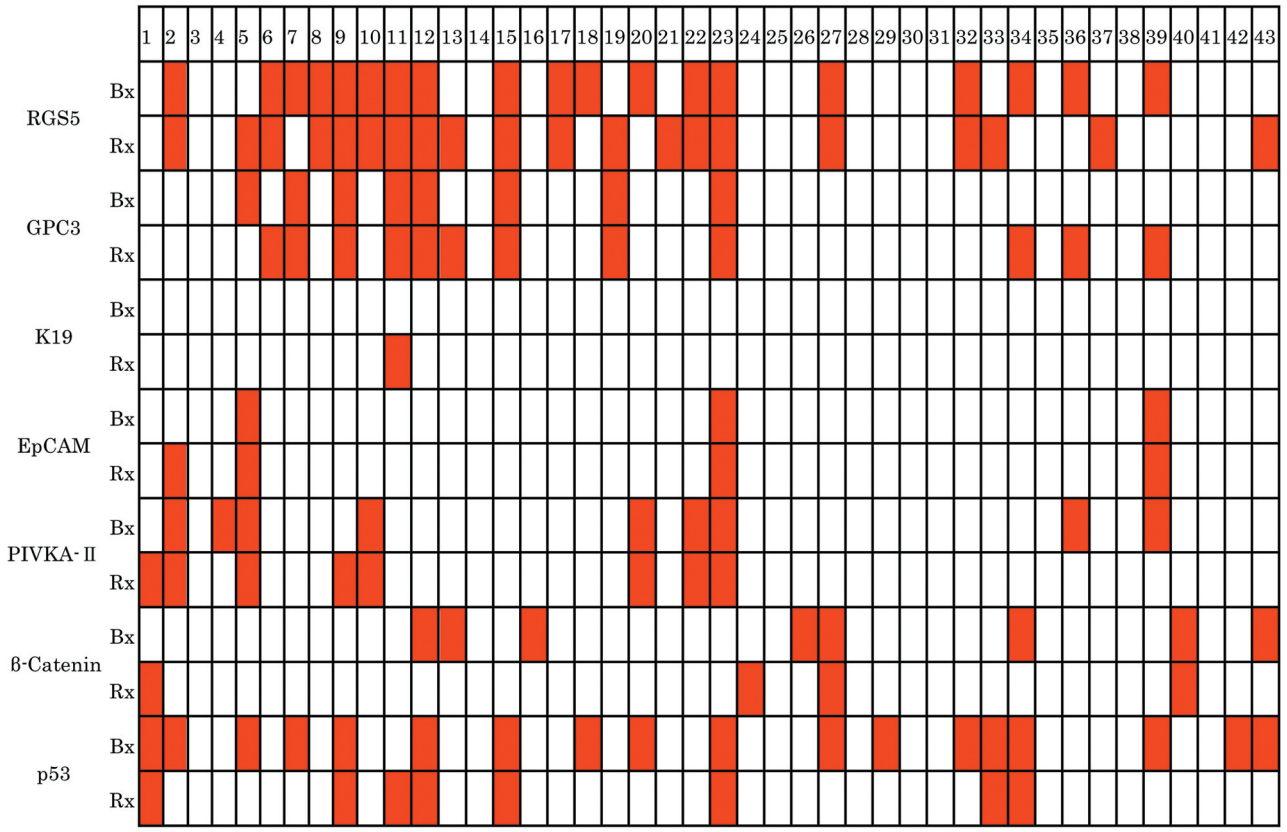

Figure 3. *Summary of the immunoprofiles in the matched preoperative biopsies and resected HCCs.*

Table I. *Summary of clinicopathological findings.*

Clinicopathological parameters	Cases (n=43)
Male/Female	28/15
Age (years)	67±9
HCV Ab-positive	31
HBs Ag-positive	7
Serum AFP (ng/ml)	274±923
Serum PIVKA-II (mAU/ml)	380±1,078
Tumor size (mm)	27±10
Differentiation (well/moderately/poorly)	
Biopsy	13/28/2
Resection	6/35/2
Histological growth pattern (thin/thick/ps/macro/solid)	
Biopsy	12/21/6/2/2
Resection	8/22/7/4/2
Portal vein invasion	
Present	23
None	20

HCV Ab: Hepatitis C virus antibody; HBs Ag: hepatitis B virus antigen; AFP: α-fetoprotein; PIVKA-II: protein induced by vitamin K absence or antagonist-II; well: well differentiated hepatocellular carcinoma; moderately: moderately differentiated hepatocellular carcinoma; poorly: poorly differentiated hepatocellular carcinoma; thin: thin trabecular type; thick: thick trabecular type; ps: pseudoglandular type; macro: macrotrabecular-massive type; solid: solid type.

Table II. *Summary of immunohistochemical findings.*

	RGS5	GPC3	K19	EpCAM	PIVKA-II	β-Catenin	p53
Sensitivity	65%	58%	–	75%	75%	50%	88%
Specificity	74%	97%	–	100%	91%	85%	69%
Accuracy	70%	86%	–	98%	88%	81%	72%

EpCAM: Epithelial cell adhesion molecule; GPC3: Glypican-3; K19: keratin 19; PIVKA-II: protein induced by vitamin K absence or antagonist-II; RGS5: regulator of G-protein signaling 5.

Discussion

In HCC treatment, specific molecular-targeted drugs including sorafenib, lenvatinib and regorafenib have been recently developed. Furthermore, new specific molecular-target drugs or immune-checkpoint inhibitors are expected to be developed. Assessment based on tumor histological growth pattern and molecular biology is important for choosing an appropriate cancer treatment for the individual. In lung cancer treatment, tumor biopsies are commonly performed to select for proper cancer pharmacotherapy drugs (8). It was reported that tumor

Table III. *Clinicopathological features and RGS5 expression in the biopsy specimens of hepatocellular carcinoma.*

	RGS5 positive (biopsy) (n=19)	RGS5 negative (biopsy) (n=24)	*p*-Value
Male/Female	12/7	16/8	n.s.
Age (years)	66±10	68±9	n.s.
HCV Ab./HBs Ag. positive	13/5	18/2	n.s.
Serum AFP (ng/ml)	550±1334	56±223	0.04
Serum PIVKA-II (mAU/ml)	521±995	268±114	n.s.
Histological findings of resected HCC			
Tumor diameter (mm)	27±2	27±2	n.s.
Well differentiated HCC	0%	25%	0.02
Moderately or poorly differentiated HCC	100%	75%	
HGP: thin trabecular type	0	8	0.005
Thick or ps type	13	16	n.s.
Macro or solid type	6	0	0.003
Portal vein invasion (Present/None)	16/3	7/17	0.0003

RGS5: Regulator of G-protein signaling 5; HCV Ab: hepatitis C virus antibody; HBs Ag: hepatitis B virus antigen; AFP: α-fetoprotein; PIVKA-II: protein induced by vitamin K absence or antagonist-II; HCC: hepatocellular carcinoma; HGP: histological growth pattern; thick: thick trabecular type; ps: pseudoglandular type; macro: macrotrabecular-massive type; solid: solid type; n.s.: not significant.

Table IV. *Clinicopathological features and GPC3 expression in the biopsy specimens of hepatocellular carcinoma.*

	GPC3 positive (biopsy) (n=8)	GPC3 negative (biopsy) (n=35)	*p*-Value
Male/Female	6/2	22/13	n.s.
Age (years)	61±9	68±9	0.04
HCV Ab./HBs Ag. positive	5/3	26/4	n.s.
Serum AFP (ng/ml)	429±847	239±947	n.s.
Serum PIVKA-II (mAU/ml)	1240±2214	184±460	0.01
Histological findings of resected HCC			
Tumor diameter (mm)	28±4	26±2	n.s.
Well differentiated HCC	0%	17%	n.s.
Moderately or poorly differentiated HCC	100%	83%	
HGP: thin trabecular type	0	8	n.s.
Thick or ps type	4	25	n.s.
Macro or solid type	4	2	0.001
Portal vein invasion (Present/None)	7/1	16/19	0.03

GPC3: Glypican-3; HCV Ab: hepatitis C virus antibody; HBs Ag: hepatitis B virus antigen; AFP: α-fetoprotein; PIVKA-II: protein induced by vitamin K absence or antagonist-II; HCC: hepatocellular carcinoma; HGP: histological growth pattern; thick: thick trabecular type; ps: pseudoglandular type; macro: macrotrabecular-massive type; solid: solid type; n.s.: not significant.

histological growth pattern and molecular biology are important to assess the biological behavior of HCC (2-4, 6). However, the utility of tumor biopsies to assess tumor histological growth pattern and molecular biology was unclear. The present study showed high rates of agreement in HCC differentiation and histological growth pattern and high levels of accuracy with regards to immunohistochemical molecular expression patterns between pairs of preoperative liver tumor biopsies and resected specimens. Our results indicate that despite the limited amount of tissue obtained through liver tumor biopsies, it may still be effective in characterizing major tumor histological features and molecular and biological characteristics.

HCC with specific morphological features such as macrotrabecular-massive is associated with poor prognosis factors including tumor size, serum α-fetoprotein levels, satellite nodules, and vascular invasion. Macrotrabecular-massive type is reported to be of high-grade malignancy. HCC, designated as macrotrabecular-massive, was defined by the presence of a predominant (>50%) macrotrabecular architecture (more than six cells thick) (6). It is still difficult to diagnose specific morphological features using clinical and imaging findings. The present study showed high rates of agreement in HCC histological growth pattern between pairs of preoperative liver tumor biopsies and resected specimens, and it is possible that specific morphological features can be evaluated using liver tumor biopsies.

Table V. *Clinicopathological features and K19 or EpCAM expression in the biopsy specimens of hepatocellular carcinoma.*

	K19 or EpCAM positive (biopsy) (n=3)	K19 and EpCAM negative (biopsy) (n=40)	*p*-Value
Male/Female	3/0	25/15	n.s.
Age (years)	55±6	68±9	0.02
HCV Ab./HBs Ag. positive	1/2	30/5	0.01
Serum AFP (ng/ml)	321±401	270±953	n.s.
Serum PIVKA-II (mAU/ml)	1967±3193	261±721	0.006
Histological findings of resected HCC			
Tumor diameter (mm)	19±6	27±2	n.s.
Well differentiated HCC	0%	15%	n.s.
Moderately or poorly differentiated HCC	100%	85%	
HGP: thin trabecular type	0	8	n.s.
Thick or ps type	2	27	n.s.
Macro or solid type	1	5	n.s.
Portal vein involvement (Present/None)	2/1	21/19	n.s.

K19: Keratin 19; EpCAM: Epithelial cell adhesion molecule; HCV Ab: hepatitis C virus antibody; HBs Ag: hepatitis B virus antigen; AFP: α-fetoprotein; PIVKA-II: protein induced by vitamin K absence or antagonist-II; HCC: hepatocellular carcinoma; HGP: histological growth pattern; thick: thick trabecular type; ps: pseudoglandular type; macro: macrotrabecular-massive type; solid: solid type; n.s.: not significant.

Table VI. *Clinicopathological features and PIVKA-II expression in the biopsy specimens of hepatocellular carcinoma.*

	PIVKA-II positive (biopsy) (n=9)	PIVKA-II negative (biopsy) (n=34)	*p*-Value
Male/Female	6/3	22/12	n.s.
Age (years)	63±11	68±9	n.s.
HCV Ab./HBs Ag. positive	6/3	25/4	n.s.
Serum AFP (ng/ml)	709±1767	159±512	n.s.
Serum PIVKA-II (mAU/ml)	1097±1882	190±665	0.02
Histological findings of resected HCC			
Tumor diameter (mm)	25±4	27±2	n.s.
Well differentiated HCC	0%	18%	n.s.
Moderately or poorly differentiated HCC	100%	82%	
HGP: thin trabecular type	0	8	n.s.
Thick or ps type	7	22	n.s.
Macro or solid type	2	4	n.s.
Portal vein involvement (Present/None)	6/3	17/17	n.s.

PIVKA-II: Protein induced by vitamin K absence or antagonist-II; HCV Ab: hepatitis C virus antibody; HBs Ag: hepatitis B virus antigen; AFP: α-fetoprotein; HCC: hepatocellular carcinoma; HGP: histological growth pattern; thick: thick trabecular type; ps: pseudoglandular type; macro: macrotrabecular-massive type; solid: solid type; n.s.: not significant.

Table VII. *Clinicopathological features and β-Catenin expression in the biopsy specimens of hepatocellular carcinoma.*

	β-Catenin positive (biopsy) (n=8)	β-Catenin negative (biopsy) (n=35)	*p*-Value
Male/Female	3/5	25/10	n.s.
Age (years)	70±13	66±9	n.s.
HCV Ab./HBs Ag. positive	7/0	24/7	n.s.
Serum AFP (ng/ml)	145±387	304±1008	n.s.
Serum PIVKA-II (mAU/ml)	40±24	458±1184	n.s.
Histological findings of resected HCC			
Tumor diameter (mm)	25±4	27±2	n.s.
Well differentiated HCC	12%	14%	n.s.
Moderately or poorly differentiated HCC	88%	86%	
HGP: thin trabecular type	1	7	n.s.
Thick or ps type	6	23	n.s.
Macro or solid type	1	5	n.s.
Portal vein involvement (Present/None)	3/5	20/15	n.s.

HCV Ab: Hepatitis C virus antibody; HBs Ag: hepatitis B virus antigen; AFP: α-fetoprotein; PIVKA-II: protein induced by vitamin K absence or antagonist-II; HCC: hepatocellular carcinoma; HGP: histological growth pattern; thick: thick trabecular type; ps: pseudoglandular type; macro: macrotrabecular-massive type; solid: solid type; n.s.: not significant.

In HCC, portal vein invasion is associated with high recurrence rates and a poor prognosis (9). Recently, we performed genetic analyses of tissues with portal vein invasion in HCC (7). We identified that RGS5 was a gene related to portal vein invasion, and the high-RGS5 expression HCC had portal vein invasion more frequently than the low-RGS5 expression HCC (7). Hu *et al*. reported that HCC cases overexpressing RGS5 were associated with higher rates of vascular invasion, HCC recurrence, and poor prognosis (10). They also reported that RGS5 overexpression may induce epithelial– mesenchymal transformation and promote tumor metastasis (10). GPC3 was shown to be specifically expressed in HCC (11, 12). The high GPC3 expression HCC has been associated with a significantly higher malignancy, increased vascular invasion, and shorter survival or disease-free survival periods (13). Tumor invasion of the portal vein peripheral branches can sometimes be difficult to assess using standard diagnostic imaging. In addition, tumor invasion of the vascular system is important in the treatment of HCC (14). Our findings suggest that IHC with RGS5 or GPC3 could be used to accurately predict portal vein invasion in tumor biopsy specimens. The ability to use liver tumor biopsy specimens for predicting portal vein invasion would be of great clinical significance. Furthermore, GPC3 is a candidate of molecular-target therapy for HCC, and clinical trials have been conducted (15). GPC3 expression is heterogeneous in HCC (16). However, in the present study we showed an overall 86% accuracy between pairs of preoperative biopsies and resected specimens. Further studies are needed to confirm whether needle tumor biopsies are suitable for evaluating GPC3 expression.

Table VIII. *Clinicopathological features and p53 expression in the biopsy specimens of hepatocellular carcinoma.*

	p53 positive (biopsy) (n=18)	p53 negative (biopsy) (n=25)	*p*-Value
Male/Female	11/7	17/8	n.s.
Age (years)	67±10	67±9	n.s.
HCV Ab./HBs Ag. positive	12/5	19/2	n.s.
Serum AFP (ng/ml)	363±1263	211±591	n.s.
Serum PIVKA-II (mAU/ml)	537±1380	267±807	n.s.
Histological findings of resected HCC			
Tumor diameter (mm)	26±3	27±2	n.s.
Well differentiated HCC	6%	20%	n.s.
Moderately or poorly differentiated HCC	94%	80%	
HGP: thin trabecular type	1	7	n.s.
Thick or ps type	12	17	n.s.
Macro or solid type	5	1	0.03
Portal vein involvement (Present/None)	12/6	11/14	n.s.

HCV Ab: Hepatitis C virus antibody; HBs Ag: hepatitis B virus antigen; AFP: α-fetoprotein; PIVKA-II: protein induced by vitamin K absence or antagonist-II; HCC: hepatocellular carcinoma; HGP: histological growth pattern; thick: thick trabecular type; ps: pseudoglandular type; macro: macrotrabecular-massive type; solid: solid type; n.s.: not significant.

K19 is a liver precursor and bile duct epithelial cell marker reported by several groups as a useful marker for detecting highly malignant HCC (17-19). K19-positive HCC is found in about 5-10% of HCC resections, with positive cases having significantly worse postresection prognoses, higher recurrence rates, and increased metastases (17-19). EpCAM has been extensively characterized as a cancer-specific surface antigen that, along with K19, is reported to be a marker of cancer stem cells in HCC (20). EpCAM expression has been shown to be an independent predictor of poor prognosis (21). In the present study, IHC with EpCAM had 98% accuracy between pairs of preoperative biopsies and resected specimens. However, the positive rates for K19 and EpCAM were low in both the biopsies and resected specimens. It has been reported that PIVKA-II-positive HCCs have worse prognosis (22). In a previous study, we found that the rates of portal vein invasion and intrahepatic metastasis were higher in PIVKA-II-positive HCC than in PIVKA-II-negative HCC (23). In this study, there is no significant difference in portal vein invasion between PIVKA-II-positive and -negative cases, although the small sample size may affect the results. β-Catenin expression has been observed in the early stages of HCC (24). However, there is no consensus regarding the relationship between β-Catenin expression by IHC and prognosis (25-27). Genetic mutations of p53 were reported to be particularly common in highly malignant HCC (28, 29). p53 genetic mutations have been reported to be associated with advanced HCC stage and poor prognosis (30, 31). In the present study, p53-positive HCCs more often showed macro trabecular-massive or solid type than p53-negative HCCs. This result indicates that the p53-positive HCCs can have a highly malignant potential. On the other hand, this result does not mean that the p53-negative HCCs have a low malignant potential, because p53-negative HCC may also contain HCCs with p53 gene deletion and HCCs with p53 gene deletion cannot be evaluated by IHC alone.

The main limitations of this study included the retrospective study design and overall relatively small sample size. While the statistical significance of the study was sufficient, the number of cases was relatively small. To determine the overall generalizability of our findings, prospective studies with an increased number of cases are required.

In conclusion, we found high rates of agreement regarding diagnoses of tumor differentiation, histological growth pattern, and immunohistochemistry between pairs of preoperative biopsies and resected specimens of HCC. These data indicate that biopsy specimens are similar in utility compared to resected specimens for assessment of the main molecular and biological characteristics of HCC tumors. Furthermore, we found that RGS5 and GPC3 immunohistochemistry of biopsy specimens may be useful for predicting portal vein invasion. Liver tumor biopsy is useful for predicting the biological behavior of HCC through histopathological and immunohistochemical findings.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Authors' Contributions

TS, Reiichiro Kondo, and HY designed the research; TS, Reiichiro Kondo, SO, JA, SM, HK, YM, MT, YK, YN, Ryoko Kuromatsu, and ON performed the research; TS analyzed the data; TS wrote the paper and HY critically revised the manuscript.

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