Soluble human leukocyte antigen-G expression in hepatitis B virus infection and hepatocellular carcinoma

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ABSTRACT

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We investigated soluble human leukocyte antigen-G (sHLA-G) expression according to the phases of hepatitis B virus (HBV) infections and hepatocellular carcinoma (HCC). A total of 267 sera from anti-HBs positive healthy individuals (n=50), chronic HBV carriers (n=45), as well as patients with active hepatitis B (n=46), liver cirrhosis (LC, n=46) and early stage HCC (n=80) were collected and assayed for sHLA-G. Relationships between sHLA-G levels and clinicopathologic parameters including HCC stages, differentiation grades, and levels of aminotransferases, HBV DNA and alpha-fetoprotein (AFP) were assessed. Concentrations of sHLA-G were higher in the active hepatitis B and HCC

groups (median sHLA-G 53.7 and 178.8 U/mL, respectively) in comparison to other groups (P<0.05), and there were no statistically significant differences among sHLA-G levels of the anti-HBs positive group, chronic HBV carrier and LC groups. Serum sHLA-G concentrations were not associated with clinicopathologic indices including the levels of aminotransferases, AFP, anti-HBs titer, HBV DNA, as well as HCC stages, numbers of tumor nodules, pathologic grades and presence of vessel invasion. The receiver operating characteristic area under the curve (AUC) value of sHLA-G for differentiating HCC from LC was 0.98, which was greater than that of AFP (0.78) (P<0.0001), and sensitivity and specificity of sHLA-G were, respectively, 90.0% and 95.7% for HCC when applying a cut-off level of 97.3 U/mL. Serum sHLA-G levels could be used as a diagnostic marker for HCC. Although sHLA-G levels did not reflect the severity of HBV infections and HCC, they were related with phases of the disease.

Key words: human leukocyte antigen-G (HLA-G), hepatocellular carcinoma (HCC), hepatitis B virus (HBV), cancer diagnosis

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I. INTRODUCTION

Hepatitis B virus (HBV) is a common pathogen causing acute and chronic hepatitis, and prolonged HBV infection can lead to liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Although, there have been many researches on hepatitis and the hepatocarcinogenesis owing to HBV infections, definite mechanisms of the host's immune tolerance, which are caused by HBV infections or may occur during hepatocarcinogenesis, are still not well-known.

Human leukocyte antigen (HLA)-G was first identified as a cell-associated and secreted class I major histocompatibility complex antigen in 1990, which was

expressed restrictively in early gestation human cytotrophoblasts and was suggested to be related with maternal tolerance to the fetus.[1] There have been many studies on the expression and function of HLA-G thereafter. Proteins coded by the *HLA-G* gene are classified into seven isoforms including four membrane-bound (HLA-G1 to -G4) and three soluble (HLA-G5 to -G7) forms according to the expressed domains from alternative splicing of the *HLA-G* transcript,[2] and membrane-bound forms can be cleaved by proteolysis and also be shed. HLA-G is known to interact with inhibitory receptors such as immunoglobulin-like transcript (ILT)-2/CD85j,[3] which is expressed on the membrane of human antigen presenting cells (APCs), T cells, and natural killer (NK) cells. It also binds to ILT-4/CD85d on the myeloid-specific APCs and killer cell immunoglobulin receptor (KIR) 2DL4 of NK and T cells.[2] In addition, soluble HLA-G (sHLA-G) can bind to CD8 co-receptor and cause apoptosis of NK and T cells.[4] HLA-G can exist in dimerized forms,[5] and HLA-G dimers have higher affinity for ILT receptors than monomers.[6] Thus, increased expression of sHLA-G can lead to immune tolerance of the host.

NK and T cell-mediated immunity is known to play important roles in the protection of the host from viral infections and cancer cell growth. HLA-G expressed by cancer cells was reported to be associated with poorer prognoses of the patients with ovarian cancers, B cell chronic lymphocytic leukemia, gastric and colorectal carcinomas.[7, 8] A recent study also suggested that plasma sHLA-G might be used as a biomarker for the diagnosis of colorectal, gastric, esophageal and lung cancer.[9] Additionally, expression of HLA-G was up-regulated in

cytotoxic T cells and monocytes in the patients with human immunodeficiency virus (HIV) infection,[10] and higher plasma levels of sHLA-G were suggested to be associated with rapid progression of HIV infections.[11, 12] A recent study reported that sHLA-G levels were considerably increased in the patients with chronic hepatitis C virus (HCV) infection when compared to those in healthy individuals.[13]

However, the relationship of sHLA-G expression with HBV infections and HCC has not yet been extensively studied. In this study, we aimed to investigate the expression of sHLA-G according to the phases of HBV infections and HCC. We also evaluated the relationship between sHLA-G levels and other clinicopathologic parameters.

II. MATERIALS AND METHODS

1. Study subjects

A total of 267 serum samples from subjects were collected during February 2010 to May 2011, and all specimens were frozen at -70 ℃ immediately after arrival until assayed. Healthy individuals with serum anti-HBs levels greater than 10 IU/L and negative HBsAg were classified into the anti-HBs positive healthy group (n=50), and patients with positive HBsAg and serum HBV DNA levels greater than 2.0×10^4 IU/mL were divided into the active hepatitis B group (n=46). The chronic HBV carrier group (n=45) consisted of subjects who had normal serum levels of aminotranferases (< 40 U/L) and were positive for HBsAg,

anti-HBc, and anti-HBe, and negative for anti-HBs and HBeAg. The LC group (n=46) comprised patients diagnosed as having LC by hepatologists using abdominal ultrasonography (U/S) and/or transient elastography (Fibroscan®).[14] For the HCC group, serum samples from eighty patients, who had been diagnosed as having HCC by computed tomography and/or abdominal U/S and who had been scheduled to undergo a surgical operation, were collected before any treatment, including systemic chemotherapy, surgery, transarterial chemoembolization, and radiofrequency ablation. After surgical operation, pathologic examination of the resected HCC tissue was performed by a pathologist specialized in hepatic neoplasms, and the results including tumor sizes, numbers of nodules, microscopic findings of vessel invasion, and differentiation grades of the tumors according to the Edmondson-Steiner's classification were recorded.[15] Cancers with pathology other than HCC (for instance, cholangiocarcinoma) were also excluded from the HCC group, and HCC stage of the patients was stratified according to the Barcelona Clinic Liver Cancer (BCLC) staging system.[16]

All the subjects except the healthy individuals in the anti-HBs positive group had current or previous history of HBV infections and any subjects with a history of alcoholic abuse, HCV infection and other infectious or systemic diseases were excluded in this study. This study was approved by the Institutional Review Board of Severance Hospital.

2. Assay for serum sHLA-G

Serum sHLA-G concentrations were measured using the Exbio/BioVendor

sHLA-G ELISA kits (Enzo Life Sciences International, Inc., Butler Pike, PA, USA). In the assay, calibrators and samples were incubated in microplate wells coated with monoclonal anti-human sHLA-G antibody. After an hour of incubation and washing steps, monoclonal anti-human β2-microglobulin antibody labeled with horseradish peroxidase (HRP) was added to the wells and incubated again for an hour. Following another washing step, the remaining HRP conjugate reacted with a substrate (tetramethylbenzidine), and the resultant reactions were read as optical densities (ODs) using an automated ELISA plate reader. Finally, sHLA-G levels (U/mL) in the specimens were calculated using the calibration curve constructed by plotting ODs against concentrations of calibrators included in the assay kits.

3. Determination of other serum markers

Tests for HBV serologic markers including HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were performed using the Abbott Architect i4000SR analyzer with respective assay kits (Abbott Laboratories, Abbott Park, IL, USA). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (U/L) in all study subjects were measured by the Hitachi 7600 DDP modular chemistry analyzer (Hitachi High-Technologies Co., Tokyo, Japan). HBV DNA viral load (IU/mL) of the patients in the active hepatitis B group was determined by Cobas AmpliPrep/Cobas TaqMan HBV Test v2.0 (Roche Molecular Systems, Inc., Pleasanton, CA, USA). AFP levels (IU/mL) were assayed by the UniCel DxI 800 Access Immunoassay System with AFP test kits (Beckman Coulter Inc., Brea,

CA, USA). Each assay was performed according to the respective manufacturer's instructions.

4. Statistical analysis

All statistical analyses were performed using the Analyse-it Method Evaluation Edition version 2.22 software (Analyse-it Software Ltd, City West Business Park, Leeds, UK). Multiple comparisons among continuous variables of the study groups were performed using the Kruskal-Wallis test with pairwise comparison and Bonferroni correction to compensate for alpha statistical errors. Continuous variables between two groups were also compared by Mann-Whitney U tests, and categorical variables of the study groups were assessed using Chi-square tests. Correlation coefficients between serum sHLA-G levels and other clinical indices were calculated using Spearman's rank tests. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performances of sHLA-G and AFP for differentiating HCC (n=80) from LC (n=46) and that of sHLA-G for discriminating active hepatitis B (n=46) from the anti-HBs positive healthy individuals (n=50), chronic HBV carriers (n=45), and both groups (n=95). The resultant area under the curve (AUC) value of sHLA-G for HCC detection was compared to that of AFP. *P*-values less than 0.05 were considered statistically significant in all analyses.

III. RESULTS

1. Characteristics of the study groups

Characteristics and the levels of serum markers in the study groups are summarized in Table 1. Ages of the subjects in all groups were different for each other group ($P<0.05$) except between the anti-HBs positive and the chronic HBV carrier groups ($P=1.0000$), between the anti-HBs positive healthy and the HCC groups ($P=0.1871$), and between the LC and the HCC groups ($P=1.0000$). There was no difference in the proportion of males in the study groups ($P=0.1055$) except between the anti-HBs positive group and all other groups.

Table 1. Characteristics of study groups

Parameter	Healthy anti-HBs positive (n=50)	Active hepatitis B (n=46)	Chronic HBV carrier (n=45)	Liver cirrhosis (n=46)	HCC (n=80)
Age (years)*	50.5 (41.9-63.1)	36.0 (30.0-48.1)	49.0 (41.7-55.3)	56.5 (51.0-63.1)	55.0 (48.0-61.0)
Male (n, %)*	14, 28.0	28, 60.9	29, 64.4	29, 63.0	63, 78.8
AST (IU/L)*	23.0 (18.9-28.0)	41.0 (23.9-76.4)	23.0 (19.0-27.3)	29.5 (23.9-41.2)	102.0 (69.0-143.8)
ALT (IU/L)*	21.5 (13.9-28.1)	51.5 (32.7-106.8)	22.0 (15.0-30.3)	31.5 (18.0-38.3)	87.5 (53.4-126.2)
HBV DNA (IU/mL)	-	$8.69x10^6$ ($4.45x10^5$-$1.70x10^8$)	-	-	-
AFP (IU/mL)	-	-	-	2.6 (1.9-3.8)	19.4 (3.2-212.0)
sHLA-G (U/mL)*	6.8 (1.5-49.6)	53.7 (3.4-293.4)	3.1 (1.5-19.6)	8.8 (1.5-28.3)	178.8 (124.4-272.3)

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; sHLA-G, soluble human leukocyte antigen-G.

Data are shown as 'median (1st to 3rd quartiles)' or numbers and percentages.

*P<0.0001 among all groups. P-values between ages of the subjects in all groups were <0.05 except between anti-HBs positive and chronic HBV carrier groups (P=1.0000), between anti-HBs positive and HCC groups (P=0.1871), and between liver cirrhosis and HCC groups (P=1.0000). There was no difference in male proportions of the study groups (P=0.1055) except the anti-HBs positive group.

Clinicopathologic characteristics of the HCC group are shown in Table 2. Most of the patients in the HCC group were stratified to BCLC stage A (73/80, 91.3%) and they also presented a single HCC nodule (70/80, 87.5%). The median tumor size was 30 mm (1st to 3rd quartiles=22 to 42 mm).

Table 2. sHLA-G levels according to the characteristics of hepatocellular carcinoma patients (n=80)

Parameter (n, %)	Value (n, %)	sHLA-G level (U/mL)	*P*-value
Gender	Male (63, 78.8)	175.6 (120.2 to 271.9)	0.9953
	Female (17, 21.3)	182.1 (130.9 to 276.6)	
BCLC stage	A (73, 91.3)	182.1 (127.3 to 273.1)	0.5414
	B (6, 7.5)	142.0 (117.6 to 219.0)	
	C (1, 1.3)	160.2 (-)	
No. of nodule	1 (70, 87.5)	181.5 (131.6 to 275.4)	0.5202
	2 (8, 10.0)	161.9 (122.6 to 220.8)	
	3 (2, 2.5)	136.1 (-)	
Edmondson-Steiner's grade	I (24, 30.0)	206.1 (133.9 to 280.8)	0.6008
	II (39, 48.8)	173.0 (120.2 to 235.2)	
	III (17, 21.3)	194.4 (118.1 to 358.1)	
	IV (0, 0)	-	
Microvessel invasion	Present (37, 46.3)	196.9 (136.8 to 286.1)	0.2334
	None (43, 53.8)	164.5 (118.1 to 269.3)	
Portal vein invasion	Present (8, 10.0)	179.5 (160.8 to 328.2)	0.5212
	None (72, 90.0)	178.8 (119.6 to 271.2)	

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; sHLA-G, soluble human leukocyte antigen-G.

Data are shown as numbers and percentages or 'median (1st to 3rd quartiles)'.

2. Levels of sHLA-G according to the study groups

Figure 1 and Table 1 show the serum levels of sHLA-G according to the study groups. Concentrations of sHLA-G were higher in the active hepatitis B and HCC groups (median sHLA-G 53.7 and 178.8 U/mL, respectively) when compared to the other groups (P<0.05), and there were no differences among the sHLA-G levels in the anti-HBs positive healthy, chronic HBV carrier, and LC groups (P>0.05).

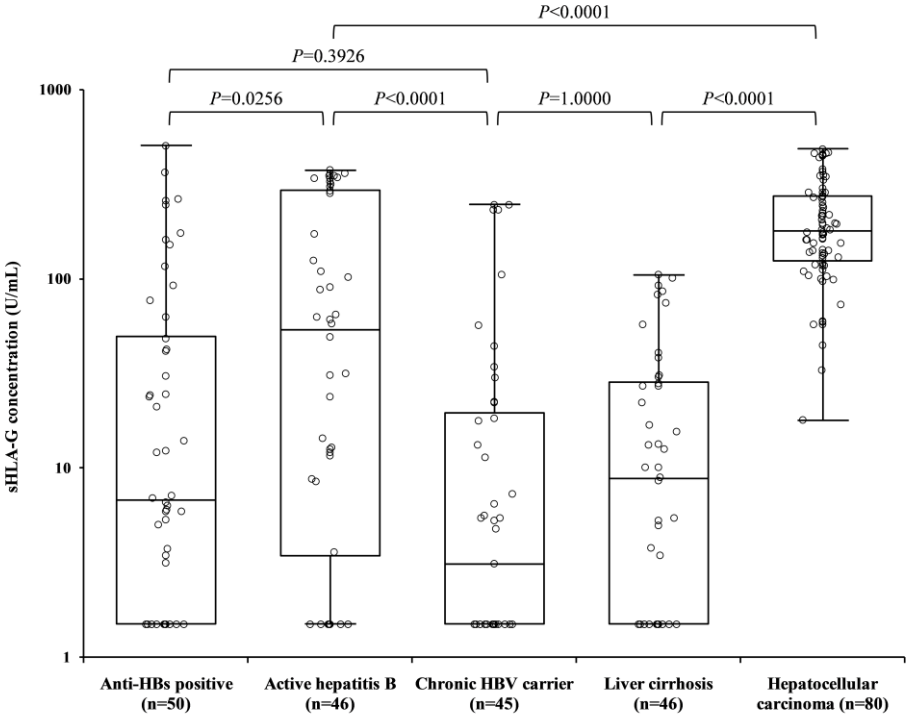

Figure 1. Serum sHLA-G levels according to the phases of hepatitis B virus (HBV) infection and early stage hepatocellular carcinoma (HCC). Expression of sHLA was increased in the patients with active hepatitis B or HCC compared to the anti-HBs positive healthy individuals, chronic HBV carriers, and the patients

with liver cirrhosis (LC). The concentration of sHLA-G was also higher in the patients with HCC than in those with LC or active hepatitis B. The upper and lower ends of boxes and box inner lines correspond to the upper and lower quartiles and median values, respectively. Whiskers denote minimum and maximum values, and circles indicate individual values.

3. Correlation of sHLA-G levels with other clinical indices

Serum sHLA-G concentrations were not significantly correlated with other clinical indices (Table 3). In each group, sHLA-G levels were not correlated with the levels of aminotransferases, except for AST levels in the anti-HBs positive (n=50, r=-0.28, P=0.0455) and the LC groups (n=46, r=0.30, P=0.0403), while there were positive correlations between the levels of sHLA-G and aminotransferases when all 267 subjects were analyzed regardless of disease phase (r=0.43 between sHLA-G and AST, r=0.37 between sHLA-G and ALT, $P<0.0001$ for both correlation coefficients). Serum anti-HBs titer (IU/L) in the anti-HBs positive group (n=50), HBV DNA levels in the active hepatitis B group (n=46) and AFP concentrations in the LC (n=46) and HCC groups (n=80) were not correlated with sHLA-G levels. Patients' ages, tumor sizes and numbers of nodules in the HCC group (n=80) also did not correspond with sHLA-G concentrations.

Table 3. Correlation of serum sHLA-G concentration with other indices

Group (n)	Parameter	*r*	*P*-value
Anti-HBs positive (50)	Anti-HBs	-0.10	0.4953
	AST	-0.28	0.0455
	ALT	-0.28	0.0500
Active hepatitis B (46)	HBV DNA	0.24	0.1107
	AST	-0.15	0.3335
	ALT	-0.14	0.3368
Chronic HBV carrier (45)	AST	-0.06	0.7153
	ALT	0.06	0.7176
Liver cirrhosis (46)	AFP	-0.07	0.6272
	AST	0.30	0.0403
	ALT	0.17	0.2609
HCC (80)	Age	-0.01	0.9608
	AFP	-0.02	0.8791
	Tumor size	-0.03	0.7706
	No. of nodules	-0.10	0.3728
	AST	0.11	0.3110
	ALT	0.16	0.1440
All (267)	AST	0.43	<0.0001
	ALT	0.37	<0.0001

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; sHLA-G, soluble human leukocyte antigen-G.

In the anti-HBs positive healthy individuals (n=50), serum sHLA-G concentrations in the anti-HBc positive subjects (n=26, median 7.1 U/mL, 1st to 3rd quartiles=3.7 to 24.2 U/mL) were not different from those in anti-HBc negative individuals (n=24, median 6.2 U/mL, 1st to 3rd quartiles=1.5 to 164.3

U/mL) (P=0.8367). In addition, there was no difference between the sHLA-G levels according to the gender of subjects, BCLC stages, numbers of tumor nodules, Edmondson-Steiner's differentiation grades, and presence of vessel invasion in the HCC group (P>0.05, Table 2).

4. Diagnostic performance of serum sHLA-G for HCC

Figure 2 illustrates ROC curves of sHLA-G and AFP for discriminating patients with HCC (n=80) from those with LC (n=46). The AUC value of sHLA-G was 0.98 (95% CI=0.96 to 1.00, *P*<0.0001). Additionally, AFP showed an AUC value of 0.78 (95% CI=0.69 to 0.86, *P*<0.0001), and there was a significant difference between the AUC values of sHLA-G and AFP (*P*<0.0001). Sensitivity and specificity of sHLA-G for differentiating HCC from LC were 90.0% (95% CI=81.2% to 95.6%) and 95.7% (95% CI=85.2% to 99.5%) when applying a cut-off level of 97.3 U/mL. AUC value of sHLA-G for detecting HCC from all other groups except the active hepatitis B group (n=141) was 0.93 (95% CI=0.90 to 0.96, *P*<0.0001), and sensitivity and specificity of sHLA-G in this case were 90.0% (95% CI=81.2% to 95.6%) and 88.7% (95% CI=82.2% to 93.4%) with the cut-off sHLA-G level of 92.5 U/mL.

In addition, AUC values of sHLA-G for discriminating active hepatitis (n=46) from anti-HBs positive healthy individuals (n=50), chronic HBV carriers (n=45), and from both groups (n=95) were 0.63 (95% CI=0.52 to 0.75, *P*=0.0105), 0.72 (95% CI=0.62 to 0.83, *P*<0.0001), and 0.68 (95% CI=0.58 to 0.78, *P*=0.0003), respectively. Sensitivity and specificity of sHLA-G for distinguishing the patients

with active hepatitis B from chronic HBV carriers were 56.5% (95% CI=41.1 to 71.1%) and 82.2% (95% CI=67.9% to 92.0%) with the cut-off level of 30.0 U/mL.

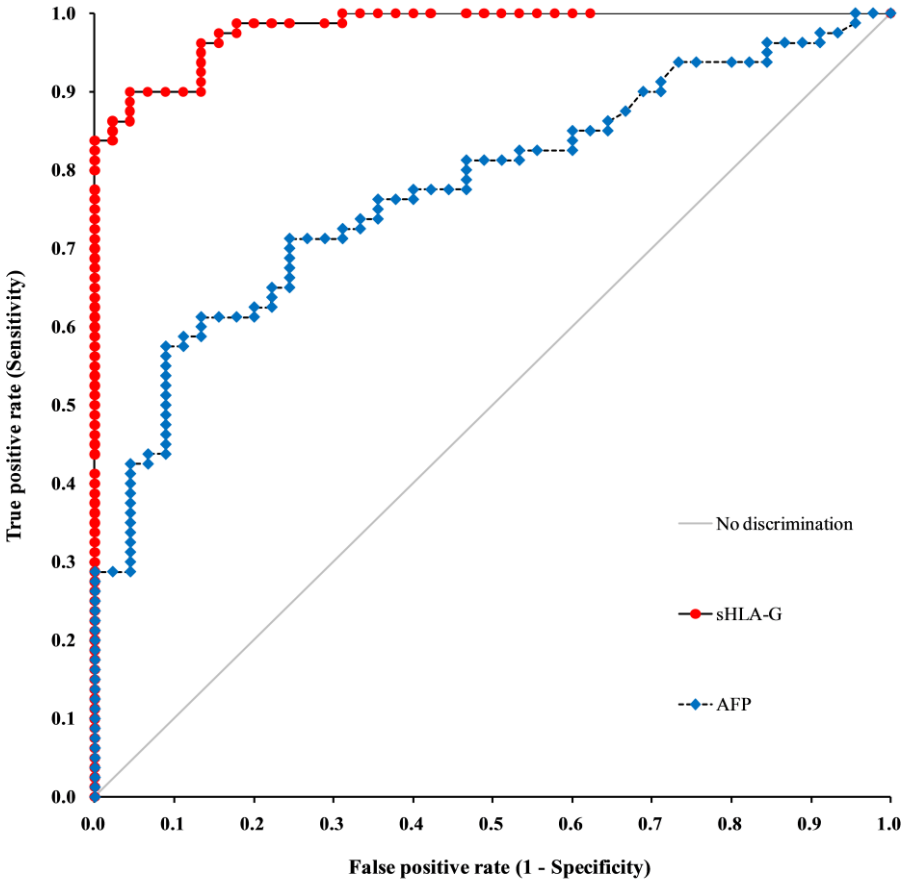

Figure 2. Diagnostic performances of the sHLA-G and alpha-fetoprotein (AFP) for discriminating patients with early stage hepatocellular carcinoma (n=80) from those with liver cirrhosis (n=46). Receiver operating characteristic - area under the curve value of sHLA-G was 0.98 (95% confidence interval [CI]=0.96 to 1.00, *P*<0.0001), and was greater than that of AFP (0.78, 95% CI=0.69 to 0.86, *P*<0.0001) (*P*<0.0001 for the difference).

IV. DISCUSSION

In this study, we investigated the expression of sHLA-G in the sera from healthy subjects and patients with HBV infections. We also compared sHLA-G concentrations in the patients with well-characterized early stage HCC. As a result, serum sHLA-G levels well correlated with the progression of HBV infections. Particularly, the patients with HCC showed the highest median sHLA-G level of 178.8 U/mL, followed by that of 53.7 U/mL in the active hepatitis B patients, among all study groups. Levels of sHLA-G were not different between the subjects in the anti-HBs positive, chronic HBV carrier, and LC groups. Similar to this finding, sHLA-G levels were higher in 36 HCC patients than those in 25 LC patients and 25 healthy individuals in a previous study, and there was no difference between the sHLA-G levels of the LC patients and healthy controls.[17] Other previous research also reported that plasma sHLA-G in HCC patients was significantly higher than that of normal controls (median, 92.5 U/ml versus 9.3 U/ml, $P< 0.001$).[18] In another recent study, sHLA-G levels were also increased in patients with acute and chronic hepatitis B (median 193.1 U/mL and 324.6 U/mL, respectively) in comparison to healthy controls (median 9.0 U/mL) and subjects with resolved HBV infection (median 14.8 U/mL), and AUC values of sHLA-G for differentiating acute and chronic hepatitis B from healthy controls were 1.000 and 0.993 with sensitivities of 97.8% and 91.6%, respectively.[19] In our data, the AUC of sHLA-G for distinguishing HCC from LC was 0.98 and was higher than that of AFP, which has been commonly used as a tumor maker for HCC. With a

cut-off of sHLA-G 97.3 U/mL, sensitivity and specificity of sHLA-G for differentiating HCC from LC were 90.0% and 95.7%. The AUC value of sHLA-G for detecting HCC from all other groups except the active hepatitis B group was also high as 0.93, and sensitivity and specificity in this case were 90.0% and 88.7% with the cut-off sHLA-G level of 92.5 U/mL. In addition, two previous studies reported shorter survival of HCC patients with high levels of tissue HLA-G when compared to those with low HLA-G tissue levels.[17, 20] Thus, the serum level of sHLA-G would be a diagnostic marker for active B-viral hepatitis and HCC and a monitoring tool for the phases of HBV infections.

Moreover, most of the HCC cases in our study had a single mass of HCC with sizes less than 50 mm and were stratified to BCLC stage A. We also confined the subjects of our study, except healthy individuals, to those having a history of B viral hepatitis and those without histories of alcohol abuse and HCV infection. These results would indicate that sHLA-G is increased during the early stage of hepatocellular carcinoma owing to HBV infection, and thus this marker may be useful in the early diagnosis of HBV related HCC; although it is not certain whether the increased expression of sHLA-G is one of the major causes of hepatocarcinogenesis and immune tolerance to HBV, or whether sHLA-G is merely produced by HCC and HBV infected hepatocytes. However, sHLA-G expression might only attribute to or be associated with the development of HCC and not with the progression and dedifferentiation of the cancer, because there were no differences between the sHLA-G levels according to the BCLC stages, numbers of tumor nodules, Edmondson-Steiner's differentiation grades, and

presence of vessel invasion in our data. The exact nature of the roles of HLA-G in hepatocarcinogenesis and progression of HCC should be investigated by further researches, since these would be a clue to the prevention and effective therapy of HCC. In any case, increased expression of sHLA-G would accelerate the development and survival of HCC, since sHLA-G is known to be related with immune tolerance of the host. In a recent study, a 14-base pair insertion polymorphism of the *HLA-G* 3' untranslated region was associated with reduced susceptibility of HBV infection and development of HCC.[21] Thus, increased expression of sHLA-G might be not a mere byproduct of HBV infected hepatocytes or HCC tissues, but may rather be a host factor, which would be associated with the regulation of immune responses to HBV infection and HCC development.

To the best of our knowledge, there are no published articles, which investigated the diagnostic performance of sHLA-G for HCC detection compared to AFP or researched the expression pattern of sHLA-G from healthy individuals and patients with active hepatitis B to those with HCC stemming from HBV infection. The relationship between sHLA-G and clinicopathologic parameters also had not yet been extensively studied. Serum sHLA-G could be increased in various conditions other than HBV infections, thus, further searches on the expression of sHLA-G in various clinical conditions such as infectious or systemic inflammatory diseases, which could be causes of increased sHLA-G expression, would be helpful to facilitate the use of sHLA-G as a diagnostic marker for HCC and active hepatitis B.

Most previous studies regarding the relationship of HLA-G with hepatitis B or HCC were performed with a small number of subjects, because HLA-G expression was investigated with HCC tissues or serum sHLA-G can only currently be measured using ELISA, which is fairly expensive and was developed only for research use. ELISA also possesses limitations of labor-intensiveness and difficulty in processing a large number of specimens. Studies with a large number of subjects and developing an automated platform for assaying sHLA-G would also be helpful to determine the clinical usefulness of sHLA-G.

Interestingly in our study, serum sHLA-G levels between the anti-HBc positive and negative subjects in the anti-HBs positive group (n=50) were not different with each other, and those in the active hepatitis B group (n=46) were not correlated with serum levels of HBV DNA and aminotransferases. The levels of sHLA-G also did not correspond to the levels of AFP and aminotransferases in the LC and HCC groups (n=46 and 80), tumor sizes, and number of HCC nodules in the HCC group (n=80). In a previous study, tissue expression intensities of HLA-G in 74 hepatitis B patients were not associated with age and gender of patients, HBeAg status, severity of liver fibrosis, and grade of histological findings.[22] Positivity of HLA-G staining in 36 HCC tissues was also not related with age and gender of patients, histological grade and stage of tumor, and AFP level.[17]. Thus, sHLA-G should not be considered as associating with the severity of HBV infections and HCC, nor past exposure to HBV, but rather only reflects the phases of the diseases including HCC caused by HBV infections.

V. CONCLUSION

Increased expression of sHLA-G could be one of the immune escape mechanisms of HBV and HCC. Expression of sHLA-G did not reflect the severity of HBV infections and HCC but were associated with the phases of the diseases caused by HBV infections. Serum sHLA-G levels would be useful in the diagnosis and monitoring of HCC and hepatitis B.

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ABSTRACT(IN KOREAN)

B형 간염과 간암에서의 용해성 사람백혈구항원-G 표현

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박 용 정

본 연구에서는 B형 간염과 간암에서의 용해성 사람백혈구항원-G (sHLA-G) 혈중 농도를 조사하였다. B형 간염항체를 보유한 건강인(n=45), 만성 B형 간염보균자(n=45), 활동성 B형 간염환자(n=46), 간경화환자(n=46) 및 초기간암환자(n=80) 등 총 267명으로부터 혈청을 수집하여 sHLA-G 농도를 측정하고, 간암의 병기, 간암조직 분화도, 간효소치, 혈중 B형 간염 바이러스 및 알파태아단백 농도 등의 임상 및 병리학적 지표들과 sHLA-G 농도의 상관관계를 분석하였다. sHLA-G 농도는 활동성 B형 간염 및 간암 환자에서 각각 중위수 53.7 U/mL 및 178.8 U/mL로 다른 연구 대상군에 비하여 높았으나(P<0.05), B형 간염항체 보유자, 만성 B형 간염보균자 및 간경화 대상군들 사이에는 차이가 없었다. 또한, sHLA-G 농도는 간효소치, 알파태아단백, B형

간염항체 및 B형 간염 바이러스 농도뿐 아니라 간암의 병기, 간암 종양의 개수, 간암의 병리학적 분화도 및 혈관 침범여부 등과 연관성이 없었다. 간암과 간경화를 구별하기 위한 표지자로서, sHLA-G는 상대수행능곡선의 곡선하 면적이 0.98로 0.78인 알파태아단백보다 진단성능이 우수하였으며(P<0.0001), 혈청내 sHLA-G 농도 97.3 U/mL를 기준으로 하는 경우 간경화군으로부터 간암을 진단하는 민감도는 90.0%, 특이도는 95.7%였다. 따라서, 혈청내 sHLA-G 농도는 간암 진단을 위한 표지자로 사용될 수 있을 것으로 생각되며, 비록 B형 간염 및 간암의 중증도를 반영하지 않았으나 sHLA-G는 B형 간염 바이러스 감염으로 기인하는 질병의 병기와 연관되어 있었다.

핵심되는 말: 사람백혈구항원-G, 간암, B형 간염 바이러스, 암진단