

Human Leukocyte Antigen-G Protein Expression Is an Unfavorable Prognostic Predictor of Hepatocellular Carcinoma following Curative Resection

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Abstract Purpose: Human leukocyte antigen-G (HLA-G) is a tumor-associated immunosuppressive molecule involved in tumor escape mechanisms. The aim of this study is to elucidate its prognostic significance in hepatocellular carcinoma (HCC).

Experimental Design: Immunohistochemical staining of HLA-G expression as well as tumor-infiltrating FoxP3⁺ regulatory (Tregs) and CD8⁺ cytotoxic T cells was carried out on tissue microarrays containing 173 HCC tissue specimens. Membrane-bound HLA-G1 protein expression in five human HCC cell lines was detected by Western blot.

Results: HLA-G expression was associated with HCC prognosis, especially in early-stage diseases, with high expression independently associated with shortened overall survival ($P = 0.041$) and increased tumor recurrence ($P = 0.023$). HLA-G level was positively related to Tregs/CD8⁺ ratio and their combination served as a better prognosticator, patients having concurrent high levels of both variables at more than three times of risk of death and tumor relapse than those with concurrent low levels (both $P < 0.001$). In addition, HLA-G1 expression increased in a concordant manner with the increase of metastatic potential in human HCC cell lines.

Conclusions: Overexpression of HLA-G protein in HCC was an independent indicator for poor outcome especially in early-stage disease. The combination of HLA-G expression and Tregs/CD8⁺ ratio added the prognostic power to both variables, offering a possible strategy of tumor-stroma interaction-oriented cancer immunotherapy.

Surgery remains to be the mainstay to prolong survival of selected patients with hepatocellular carcinoma (HCC). However, the major hurdle for outcome following resection is tumor recurrence, which may even exceed 70% at 5 years (1), although researchers have endeavored for decades for the precise prediction of HCC recurrence, from determination of various clinicopathologic factors (2), to the alterations in oncogenes/oncogenic pathways or suppressor genes (3, 4), to

the immune components of tumor microenvironment (5–7), and to the latest gene expression signatures (8–10). The applications of existing makers are yet unsatisfactory and the prognosis of HCC is still grave. From an immunobiologic perspective, tumor local immune response comprises two arms: antitumor immunity such as CD8⁺ T cells, natural killer (NK) cells, and NK T cells and protumor factors such as regulatory T cells (Tregs) and tumor-derived repressive factors (11). The balance between antitumor and protumor factors is important for tumor recurrence. Our previous study showed that a balance toward Tregs indicated weak antitumor activities and impaired outcome and vice versa (5). To explain the dual roles of local immune response, researchers raised the new concept of “immunoediting,” comprised by three phases: elimination (i.e., immunosurveillance), equilibrium, and escape (12). Recently, the escape mechanisms used by tumor cells attracted much more attention (11, 13). The immunosuppressive network at tumor microenvironment, mainly composed by tumor-associated factors and Tregs, is crucial to tumor progression (11), a more accurate molecular classification, a precise prognosticator, and the exploration of more effective therapeutic regimens (14). Our recent study revealed that programmed death ligand 1, a negative costimulatory signal expressed on tumor cells, was significantly associated with tumor aggressiveness and postoperative recurrence in HCC (15). Nevertheless, certain tumor-derived immunosuppressive factor (16, 17) even showed benefits to patients’ outcome in HCC (18). Large-scale studies with comprehensive clinical data illustrating

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Translational Relevance

Hepatocellular carcinoma is a lethal disease with high frequencies of postsurgical recurrence and is highly refractory to existing adjuvant therapies. Recent years have witnessed the renaissance of tumor immunosurveillance concept and the expansion of the initial notion into “immunoeediting,” among which the tumor escape phase attracts the most interest of researchers. Human leukocyte antigen-G (HLA-G) is a tumor-derived immunosuppressive molecule. We showed for the first time that overexpression of HLA-G protein impaired hepatocellular carcinoma outcome, especially in early-stage patients. Moreover, HLA-G expression was positively associated with tumor-infiltrating regulatory/effector T-cell ratio, another immunosuppressive indicator, and their combination was a superior prognosticator. Thus, the combination of markers derived from tumor and stroma provides a comprehensive prognostic indicator and a possible tumor-stroma interaction-oriented cancer immunotherapy.

tumor-derived molecular immunosuppressive factors in HCC are scanty. Hence, further intensive experiments of those factors are substantial.

Human leukocyte antigen-G (HLA-G) is a nonclassical MHC class I molecule, expressed as seven isoforms, including four membrane-bound (HLA-G1 to HLA-G4) and three soluble (HLA-G5 to HLA-G7) forms (19). Its restricted distribution in normal tissue and aberrant expression in pathologic conditions, such as tumor and virus-infected cells, suggest that microenvironmental factors control HLA-G expression in injured cells and tissues (19). It has emerged as a tumor-derived immunosuppressive molecule with the following abilities: (a) inhibitory function against NK cells, T lymphocytes, and antigen-presenting cells through direct binding to the inhibitory receptors (19) or through “troglodytosis” (a fast uptake of membranes and associated molecules from one cell by another; ref. 20); (b) apoptotic effects on activated CD8⁺ T cells after soluble form binding to CD8 and triggering a Fas/FasL-dependent pathway (21); (c) inducing the shift toward Th2 cytokine profile that in return up-regulates HLA-G expression at the tumor site (21); and (d) induction of Tregs (reviewed in refs. 19, 21). As a result of its panoply of immune suppression, overexpression of HLA-G protein in colorectal cancer (22), non-small cell lung cancer (23), and gastric cancer (24) is associated with poor prognosis. However, contrasting results exist even in patients with the same tumor type, such as gastric cancer (24, 25) and B-cell chronic lymphocytic leukemia (26, 27).

About the role of HLA-G in HCC, no related studies have been carried out yet. Thereby, this study is designed to elucidate the significance of HLA-G expression in HCC. We also evaluated the relations between HLA-G and Tregs/CD8⁺ ratio, which was also an indicator of local immune suppressive status. We depicted that HLA-G protein was an unfavorable predictor in HCC especially in early HCC. A positive correlation between HLA-G expression and Tregs/CD8⁺ ratio was observed and their combination served as a better predictor than either of them alone.

Materials and Methods

Patient cohort and clinical follow-up. According to the selection criteria described in our previous studies (15), 2,415 consecutive patients who underwent resection for HCC at Zhong Shan Hospital between January 2002 and September 2006 were identified as an unselected cohort. One hundred eighty patients with comprehensive clinicopathologic data were randomly selected as the study cohort after approval from the Institutional Review Board and informed consent.

The Barcelona Clinic Liver Cancer (BCLC) staging system was applied to classify the disease stage (28). The histologic grade of tumor differentiation was determined by the Edmondson-Steiner grading system. Liver function was assessed by the Child-Pugh score system.

Survival data, including survival time and time to recurrence (TTR; ref. 29), were determined on September 30, 2008. Overall survival (OS) was taken as date from primary surgical treatment to death or the last follow-up date. TTR was defined as the interval from primary surgical treatment to the first recurrence. Postoperative treatments and surveillance were carried out according to a uniform protocol as described in our previous studies (5, 7).

Tissue microarrays and immunohistochemistry. Tissue microarray blocks were constructed as previously described (5). In brief, for each case, we sampled three tissue cores of tumor at 1 mm in diameter together with different controls to construct the microarrays.

Immunohistochemical analysis of 4- μ m tissue microarray sections was carried out as previously described (5, 15). A standard two-step protocol (Novolink Polymer Detection System) was applied with the following primary antibodies: mouse anti-human FoxP3 (1:100; AbD Serotec), HLA-G (clone MEM-G/1, 1:100; Santa Cruz Biotechnology), and CD8 (1:50; Dako).

Cell lines. Five human HCC cell lines were used, including four HCC cell lines (MHCC97L, MHCC97H, HCCLM3, and HCCLM6) from the same parental cell line with stepwise metastatic potential (30), initially established and preserved by our institute, and a nonmetastatic human HCC cell line (Hep3B) purchased from the American Type Culture Collection. All cell lines were cultured in DMEM high-glucose medium with 10% fetal bovine serum at humidified 37°C incubator supplied with 5% CO₂.

Western blot analysis. Cell lysis and protein extracts were done using methods described previously (31). Lysates were separated by SDS-PAGE, and detection of membrane-bound HLA-G1 expression was done with mouse anti-human monoclonal antibody MEM-G/1 (1:500; Santa Cruz Biotechnology). Protein loading was assessed using anti- β -actin antibody (1:10,000). The ratio of HLA-G1 and β -actin was calculated as the relative quantitation. The results presented were representative of three experiments as previously described (15).

Evaluation of immunohistochemical staining. The density of HLA-G-positive staining was evaluated using a computerized image system, including a Leica DFC420 charge-coupled device camera and a Leica DM IRE2 microscope (Leica Microsystems Imaging Solution Ltd.). The capture of the photographs and measurement of HLA-G-positive staining density were described in detail previously (15, 32). In brief, three representative fields of each case were captured by the Leica QWin Plus v3 software under identical settings and magnification (\times 200). The integrated absorbance and the area in a photograph were measured by Image-Pro Plus v6.0 software (Media Cybernetics, Inc.). A uniform setting of color segmentation was loaded for counting the integrated absorbance of all the pictures. The mean HLA-G density was calculated as the product of integrated absorbance/total area.

The manual count of tumor-infiltrating lymphocytes (TIL) was described in our previous reports (5). The entire disc of each triplicate was selected for enumeration.

Statistics. Statistical analyses were carried out using Statistical Package for the Social Sciences 15.0 (SPSS). χ^2 test, Fisher's exact test, and Spearman's rank correlation were done as appropriate. Univariate analyses were done using the Kaplan-Meier method and compared by

the log-rank test. Cox multivariate analysis applied in a stepwise backward likelihood ratio method was used to adjust for potentially confounding variables and to determine the independent prognostic factors. Receiver operating curve (ROC) analysis was used to compare the prognostic value of the parameters. *P* values (two sided) of <0.05 were considered statistically significant.

The optimal cutoff for dichotomizing HLA-G expression data was determined using X-tile 3.6.1 software (Yale University of New Haven; refs. 33, 34). The median values of FoxP3⁺ Tregs, CD8⁺ TILs, and their ratio were used as cutoffs.

Results

Characteristics of the patient cohort. From the 180 cases of the tissue microarray, 7 cases (3.9%) were excluded due to tissue loss. The clinicopathologic characteristics of 173 patients are shown in Supplementary Table S1. At last follow-up, 82 (47.4%) patients had died of recurrence (*n* = 39) or cirrhosis-

related complications without recurrence (*n* = 43). The 1-, 3-, and 5-year OS were 84%, 55%, and 46%, respectively. Among the remaining 91 patients, the mean duration of follow-up was 37.2 months (range, 23.0-77.5 months; SD, 13.6). Altogether, 88 patients (50.9%) had recurrence, including intrahepatic recurrence (*n* = 63), distant metastasis (*n* = 13), or both (*n* = 12). Tumor recurrence probabilities at 1, 3, and 5 years were 35%, 52%, and 59%, respectively.

HLA-G expression and patient outcome. The expression pattern of HLA-G protein in tumor cells seemed to be very diffuse in most cases. The staining of HLA-G was mainly cytoplasmatic (Fig. 1A and B). Of the 173 tumors, 74 (43%) were ranked as low and 99 (57%) as high HLA-G expression as determined by the X-tile software.

On univariate analysis, higher HLA-G expression displayed relevance to poorer OS compared with low expression (median, 31.0 versus 63.5 months; *P* = 0.024; Fig. 2A; Table 1). The

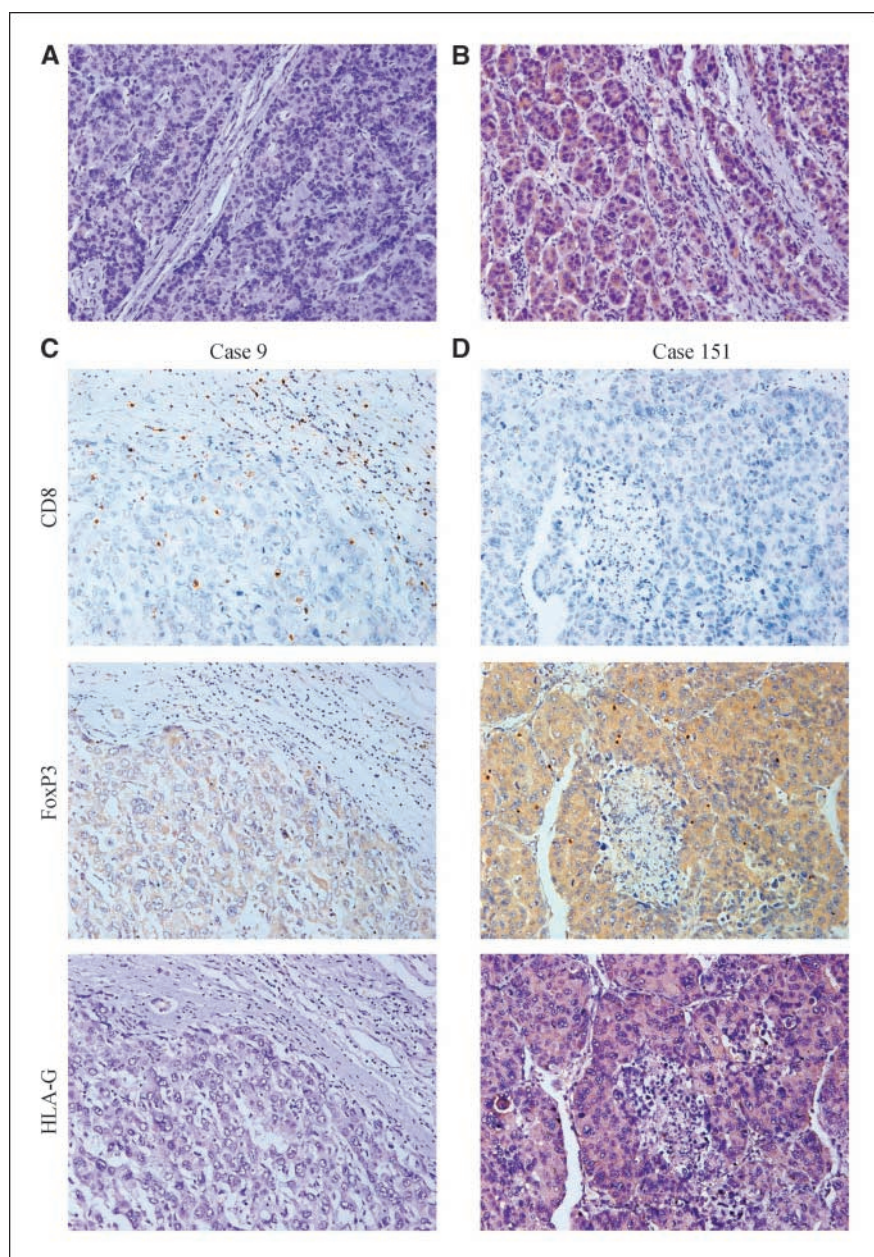


Fig. 1. HLA-G expression in HCC tissue samples and its relation with Tregs and CD8⁺ cells. Representative HCC tumor specimens with low (A) and high (B) expression of HLA-G protein by immunohistochemical staining. C, consecutive sections of case 9 showed high CD8⁺ TILs, low Tregs, and low HLA-G staining. D, consecutive sections of case 151 showed low CD8⁺ TILs, high Tregs, and high HLA-G staining. Magnification, ×200.

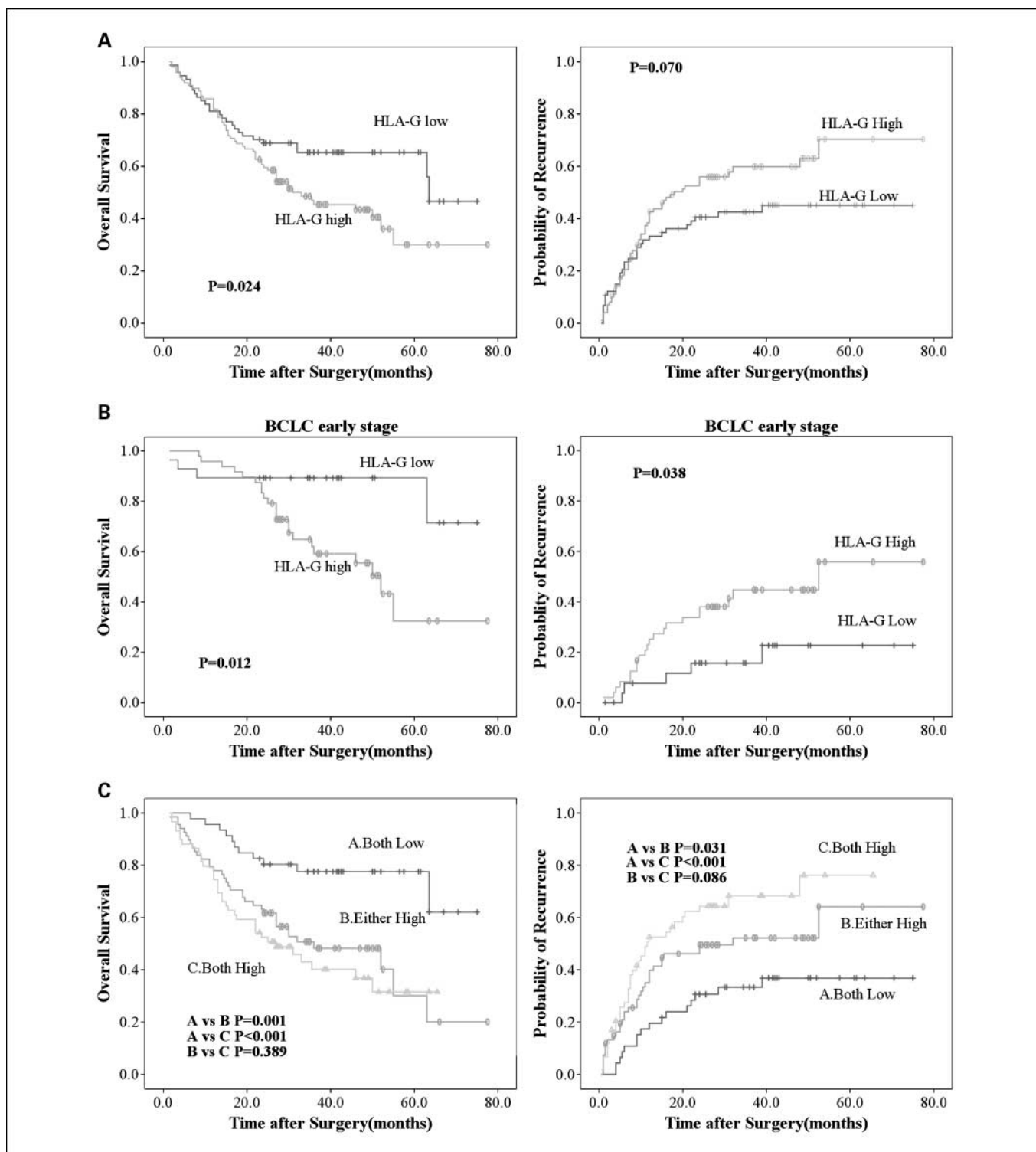


Fig. 2. Kaplan-Meier curves of survival differences among HCC patients. Differences of OS and TTR between HLA-G high and low groups in all population (A) and in early-stage patients (B). C, illustrated OS and TTR differences in combined group of HLA-G expression and Tregs/CD8⁺ ratio.

univariate analysis also found a trend toward TTR decrease, with the median time not reached versus 18 months for low and high HLA-G groups, respectively ($P = 0.070$; Fig. 2A). Multivariate analysis showed that HLA-G expression was independently associated with OS [hazard ratio (HR), 1.987; 95% confidential interval (95% CI), 1.241-3.180; $P = 0.004$;

Table 1]. Besides, BCLC stage, α -fetoprotein (AFP), and γ -glutamyl transferase level were also independent survival predictors ($P = 0.001$, 0.024, and 0.002, respectively). Early BCLC stage and complete tumor encapsulation were independently associated with decreased recurrence rate ($P < 0.001$ and $P = 0.015$, respectively).

Because BCLC stage was significant in multivariate analysis of both OS and recurrence and represented well the different stages of tumor progression, subgroup analysis was done for a comprehensive knowledge about HLA-G function within different stages. Patients with high HLA-G expression possessed shortened survival (52 months versus not reached, $P = 0.012$) and significantly accelerated recurrence (52.5 months versus not reached, $P = 0.038$) compared with those with low level in early HCC (BCLC stage 0 + A; Fig. 2B). In multivariate analysis, high HLA-G group was at high risk of poorer survival and recurrence in early HCC patients (HR, 3.145; 95% CI, 1.051-9.418; $P = 0.041$ and HR, 3.208; 95% CI, 1.176-8.750; $P = 0.023$, respectively; Table 1). The results in intermediate and advanced stages were not inspiring.

HLA-G expression in human HCC cell lines. A 38-kDa band corresponding to membrane-bound HLA-G1 protein was detected in human HCC cell lines. Strikingly, HLA-G1 expression displayed a concordant elevation with their stepwise metastatic potentials in cell lines MHCC97L, MHCC97H, HCCLM3, and HCCLM6 (Fig. 3; ref. 30). HLA-G1 protein shown in nonmetastatic cell line Hep3B was very weak (Fig. 3).

Correlations among HLA-G expression, clinicopathologic parameters, and TILs. HLA-G protein levels were found independent of other clinicopathologic parameters except for gender ($P = 0.036$; Supplementary Table S2). No correlation between HLA-G expression and Tregs was found, nor with CD8⁺ TILs. Intriguingly, a positive correlation was discovered between tumor HLA-G expression and Tregs/CD8⁺ ratio ($r = 0.193$; $P = 0.012$), with the high HLA-G patients showing

higher Tregs/CD8⁺ ratios compared with low HLA-G patients ($P = 0.013$, Mann-Whitney U test; Figs. 1C and D and 4). High Tregs/CD8⁺ ratio was in close association with larger tumor size ($P = 0.012$), vascular invasion ($P = 0.002$), elevated AFP levels ($P = 0.003$), and advanced BCLC stage ($P = 0.001$; Supplementary Table S3).

Significance of combination of HLA-G expression and Tregs/CD8⁺ ratio and ROC analysis. Tregs/CD8⁺ ratio was also an adverse indicator of patient outcome. Generally, patients with high Tregs/CD8⁺ ratio were more prone to tumor recurrence and decreased survival. Specifically, Tregs/CD8⁺ ratio was independently associated with recurrence in advanced-stage HCC (HR, 3.475; 95% CI, 1.510-7.998; $P = 0.003$) other than early or intermediate stage. Given their statistical correlation, functional interactions (35, 36), and both showing adverse prognostic significance, the power of HLA-G-Tregs/CD8⁺ ratio combination for predicting patient outcome was evaluated. Patients were further divided into three groups according to this combination, namely, group A (both low, $n = 46$), group B (either high, $n = 68$), and group C (both high, $n = 59$). When compared with group B or C, group A revealed significant higher OS ($P = 0.001$ and $P < 0.001$, respectively) and longer TTR ($P = 0.031$ and $P < 0.001$, respectively; Fig. 2C). Furthermore, this combination retained its significance in multivariate analysis for both OS ($P = 0.001$) and TTR ($P < 0.001$). Specifically, when group C was compared with group A, the corresponding HRs in multivariate analysis were 3.541 (95% CI, 1.777-7.056; $P < 0.001$) for death and 3.050 (95% CI, 1.676-5.548; $P < 0.001$) for recurrence, which were higher than

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Table 1. Univariate and multivariate analysis in total patients and early stage

Variables	OS			TTR		
	Univariate P	Multivariate analysis		Univariate P	Multivariate analysis	
		HR (95% CI)	P		HR (95% CI)	P
Early stage						
Tumor number (single vs multiple)	NS	NA	NA	0.037	NA	NS
Tumor encapsulation (complete vs no)	NS	NA	NA	0.020	NA	NS
ALT level, units/L (≤ 40 vs > 40)	0.013	NA	NS	0.037	NA	NS
Vascular invasion (no vs yes)	< 0.001	3.683 (1.547-8.771)	0.003	< 0.001	10.264 (3.274-32.174)	< 0.001
AFP level, ng/mL (≤ 20 vs > 20)	0.013	2.776 (1.233-6.249)	0.014	0.036	5.147 (1.913-13.847)	0.001
HLA-G (low vs high)	0.012	3.145 (1.051-9.418)	0.041	0.038	3.208 (1.176-8.750)	0.023
All patients						
Tumor number (single vs multiple)	NS	NA	NA*	0.015	NA	NA*
Tumor size, cm (≤ 5 vs > 5)	< 0.001	NA	NA*	< 0.001	NA	NA*
Vascular invasion (no vs yes)	< 0.001	NA	NA*	< 0.001	NA	NA*
Tumor encapsulation (complete vs no)	0.033	NA	NS	< 0.001	1.778 (1.118-2.829)	0.015
BCLC stage (0 + A vs B vs C)	< 0.001	1.577 (1.200-2.072)	0.001	< 0.001	1.852 (1.424-2.409)	< 0.001
AFP level, ng/mL (≤ 20 vs > 20)	0.001	1.816 (1.082-3.048)	0.024	0.003	NA	NS
γ -GT, units/L (≤ 54 vs > 54)	< 0.001	2.176 (1.328-3.567)	0.002	0.013	NA	NS
HLA-G (low vs high)	0.024	1.987 (1.241-3.180)	0.004	0.070	NA	NA
Tregs/CD8 ⁺ ratio (low vs high) [†]	< 0.001	2.005 (1.261-3.188)	0.003	< 0.001	2.121 (1.367-3.292)	0.001
Combination [‡] of HLA-G and Tregs/CD8 ⁺ ratio (group A vs B vs C [‡])						
Overall	< 0.001	NA	0.001	< 0.001	NA	< 0.001
B vs A	0.001	3.407 (1.712-6.778)	< 0.001	0.031	2.511 (1.366-4.617)	0.003
C vs A	< 0.001	3.541 (1.777-7.056)	< 0.001	< 0.001	3.050 (1.676-5.548)	< 0.001

NOTE: Variables with $P < 0.05$ in univariate analysis were enrolled in multivariate analysis.

Abbreviations: ALT, alanine aminotransferase; γ -GT, γ -glutamyl transferase; NA, not assessed; NS, not significant.

*Variables included in BCLC stage system were not assessed in multivariate analysis to avoid duplication.

[†]Variables were analyzed in separate multivariate analyses as not to influence each others' HR.

[‡]Combination of HLA-G and Tregs/CD8⁺ ratio: group A (both low levels of HLA-G and Tregs/CD8⁺ ratio, $n = 46$), group B (either high level of HLA-G and Tregs/CD8⁺ ratio, $n = 68$), and group C (both high levels of HLA-G and Tregs/CD8⁺ ratio, $n = 59$).

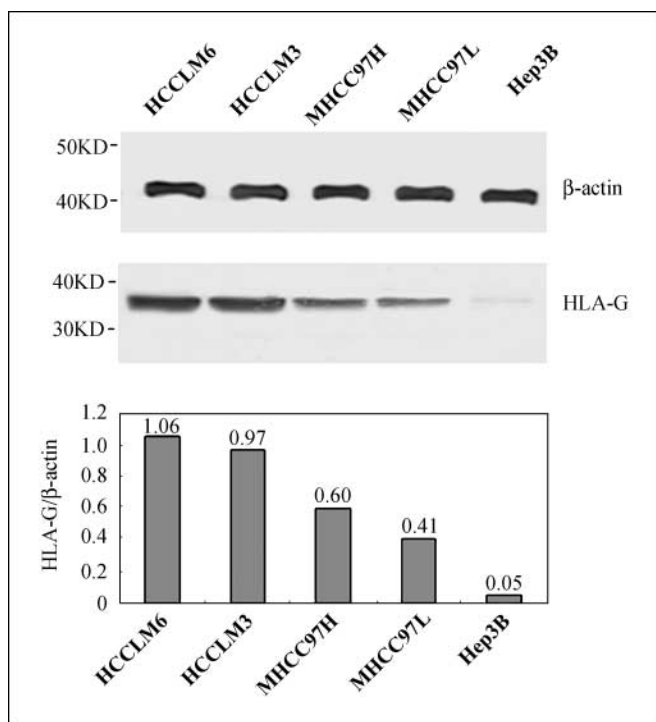


Fig. 3. HLA-G expressions in human HCC cell lines. Western blot detected membrane-bound HLA-G1 (38 kDa) expression in five human HCC cell lines. The relative quantitation was also shown.

those of HLA-G or Tregs/CD8⁺ alone (Table 1). ROC analysis revealed that the combination outdone either Tregs/CD8⁺ ratio or HLA-G alone, with an area under the curve of 0.642 versus 0.619 versus 0.594 for death and 0.624 versus 0.607 versus 0.577 for recurrence (Table 2).

Discussion

Herein, we reported for the first time that high HLA-G expression was associated with reduced survival and increased recurrence in HCC. The prognostic value of HLA-G elucidated in this study is concordant with the main stream of previous observations from other cancers (22–24). Albeit HLA-G group was only significant in OS by multivariate analysis in all patient population, its independent power was enhanced in predicting both survival and recurrence in early HCC patients indicated by a sharply increased HR (3.145 versus 1.987) for death and a gained significance for recurrence. Its independent prognostic significance in early HCC patients is of clinical importance because among intermediate to advanced stages of HCC, multinodularity (37) and the presence of vascular invasion (38) have already been well-established adverse prognostic predictors after liver resection, while the prognosis of early-stage HCC is far from homogenous and its lack of those clinicopathologic indicators and existing classification systems have shown limitations on precise prediction of patients' outcome (39). With the shift of more patients diagnosed at earlier stages, establishment of novel prognostic markers is more important for early than advanced stages and our results meet this requirement to refine the prognosis of early-stage patients. The varied predictive value between different stages of HCC may also indicate the complicated role of HLA-G in the

dynamic process of cancer immunoeediting (40). From a therapeutic view, it suggests that molecular therapies targeting at HLA-G in early-stage HCC could possibly block tumor progression.

A positive correlation was unraveled between HLA-G and Tregs/CD8⁺ ratio. Coincidentally, both of them had adverse effect on patients' outcome and were related to dampening the antitumor immunity in tumor microenvironment (19, 41). Taking together their functional interactions (35, 36), we evaluated the prognostic power of HLA-G expression and Tregs/CD8⁺ ratio. This combination was independently related to survival and recurrence of HCC patients. Patients with concurrent high levels of Tregs/CD8⁺ ratio and HLA-G expression were at more than three times of risk of death and recurrence than concurrent low levels ones. Moreover, its predictive power was superior to that of HLA-G or Tregs/CD8⁺ ratio alone on ROC analysis. These observations gave us a clue that there existed a synergy between HLA-G and Tregs/CD8⁺ ratio, and this might be attributed to the capacity of HLA-G on TIL regulation to dampen the antitumor activities. Intensive *in vitro* experiments supported the following hypothesis: (a) HLA-G can induce Tregs through distinct processes (19) and Tregs can further impair CD8⁺ T-cell functions (41) and (b) soluble HLA-G induces apoptosis in T and NK CD8⁺ cells and inhibits cytotoxic T-cell activity through CD8 ligation (35). More importantly, through our stratified analysis, we found that either HLA-G or Tregs/CD8⁺ ratio was independently associated with TTR in specified groups (i.e., early HCC or advanced HCC, respectively). When they were combined, their predictive power was independent of HCC stages. Therefore, we propose that the decisive component of tumor escape mechanisms might shift from tumor-derived factors to host-related factors (e.g., from HLA-G to Tregs/CD8⁺ ratio) during the progression of HCC according to the variance of their prognostic power in different tumor stages. However, the three steps of immunoeediting are an interdependent other than an independent process (42). Thus, the combination of HLA-G and Tregs/CD8⁺ ratio added to our knowledge of how different escape mechanisms acted and interacted, meanwhile offering a more precise look at the immune status and resulting in a better predictive indicator.

In our *in vitro* observation, the protein load detected in Western blot analysis was consistent with the metastatic

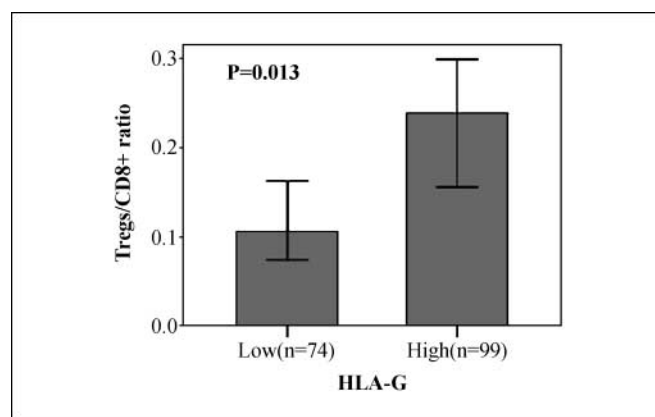


Fig. 4. Distribution of Tregs/CD8⁺ ratios between HLA-G groups. Patients with high HLA-G expression have a significantly higher Tregs/CD8⁺ ratio than low HLA-G patients. *P* value was assessed by Mann-Whitney *U* test.

Table 2. ROC analysis in all patients

Variables	Survival		Recurrence	
	AUC	SE (95% CI)	AUC	SE (95% CI)
Tregs/CD8 ⁺ ratio	0.619	0.043 (0.535-0.703)	0.607	0.043 (0.523-0.691)
HLA-G	0.594	0.043 (0.509-0.678)	0.577	0.044 (0.492-0.662)
Combination*	0.642	0.042 (0.560-0.724)	0.624	0.042 (0.541-0.707)
BCLC stage	0.629	0.042 (0.546-0.712)	0.677	0.041 (0.597-0.757)

Abbreviation: AUC, area under the curve.

*Combination of HLA-G and Tregs/CD8⁺ ratio.

potentials in the stepwise metastatic model from the same parental cell line. Protein level detected in the nonmetastatic cell line Hep3B was very low. However, no correlation was discovered between HLA-G level and tumor biologic behaviors in present *in situ* study. The discrepancy between HLA-G expression in surgically removed lesions and in cell lines indicated that stromal factors presented in tumor but not *in vitro* cell lines were substantial to maintain HLA-G expression. This was not a fortuitous phenomenon, for similar results are being observed in renal cell carcinoma (31, 43) and in melanoma (44, 45). Plenty of microenvironmental factors have already been recognized to influence HLA-G expression, including stress, hypoxia, and cytokines such as granulocyte macrophage colony-stimulating factor, IFNs, interleukin-10, and tumor necrosis factor- α (reviewed in ref. 19). These results further underscore the significance of comprehensive study on both tumor-derived molecules and host immune factors to obtain a better prognostic indicator.

Considering its prognostic value and effect on immune cells, using HLA-G as a target molecule in specific immunotherapy against tumor has already been under consideration. Most recently, a pilot study was done to use HLA-G₁₄₆₋₁₅₄ peptide as a promising candidate in peptide-based immunotherapy for HLA-A24⁺ renal cell carcinoma (46).

In conclusion, we have shown that overexpression of HLA-G in HCC was a significant independent prognosticator for poor outcome especially in early-stage diseases. The prognostic superiority of the combination of HLA-G expression and Tregs/CD8⁺ ratio conferred that the combined efforts on HLA-G blockade, Treg depletion, and cytotoxic T-cell stimulation might be promising, representing a combined tumor-stroma-targeted cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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