Genomics & Informatics Vol. 6(4) 192-201, December 2008

Erythropoietin-producing Human Hepatocellular Carcinoma Receptor B1 Polymorphisms are Associated with HBV-infected Chronic Liver Disease and Hepatocellular Carcinoma in a Korean Population

Kyoung-Yeon Kim[1], Seung Ku Lee[1], Min-Ho Kim[1], Jae Youn Cheong[2], Sung Won Cho[2], Kap-Seok Yang[3] and KyuBum Kwack[1]*

[1]Medical Genomics Laboratory, Graduate School of Life Science and Biotechnology, Pochon CHA University, Seongnam 463-836, Korea, [2]Department of Gastroenterology, Genomic Research Center for Gastroenterology, Ajou University School of Medicine, Suwon 442-721, Korea, [3]Macrogen Inc. 60-24, Gasan-dong, Gumcheon-gu, Seoul 153-781, Korea

Abstract

Erythropoietin-producing human hepatocellular carcinoma receptor B1 (EPHB1) is a member of the Eph family of receptor tyrosine kinases that mediate vascular system development. Eph receptor overexpression has been observed in various cancers and is related to the malignant transformation, metastasis, and differentiation of cancers, including hepatocellular carcinoma (HCC). Eph receptors regulate cell migration and attachment to the extracellular matrix by modulating integrin activity. EphrinB1, the ligand of EPHB1, has been shown to regulate HCC carcinogenesis. Here, we sought to determine whether EPHB1 polymorphisms are associated with hepatitis B virus (HBV)-infected liver diseases, including chronic liver disease (CLD) and HCC. We genotyped 26 EPHB1 single nucleotide polymorphisms (SNPs) in 399 Korean CLD, HCC, and LD (CLD+HCC) cases and seroconverted controls (HBV clearance, CLE) using the GoldenGate assay. Two SNPs (rs6793828 and rs11717042) and 1 haplotype that were composed of these SNPs were associated with an increased risk for CLD, HCC, and LD (CLD+HCC) compared with CLE. Haplotypes that could be associated with HBV-infected liver diseases by affecting downstream signaling were located in the Eph tyrosine kinase domain of EPHB1. Therefore, we suggest that EPHB1 SNPs, haplotypes, and diplotypes may be genetic markers for the progression of HBV-associated acute hepatitis to CLD and HCC.

Keywords: chronic liver disease, hepatocellular carcinoma, Eph receptor, angiogenesis, polymorphism

*Corresponding author: E-mail kbkwack@cha.ac.kr, kbkwack@gmail.com
Tel +82-31-725-8393, Fax +82-31-725-8350
Accepted 19 November 2008

Introduction

The single nucleotide polymorphism (SNP) is the most common genomic sequence variation and involves the stable substitution of a single base in the human genome. It was recently reported that various SNPs may increase susceptibility to risk factors for the development of a variety of cancers (Storey *et al.*, 1998; Calin *et al.*, 2005; Kiyohara & Yoshimasu, 2007; Dutt & Beroukhim, 2007; Zeng *et al.*, 2006). Moreover, SNPs in various genes, such as the genes for cell division cycle protein 6 (Cdc6) (Xiong *et al.*, 2008), patched homolog (PTCH) (Fu *et al.*, 2008), interleukin (IL)-10, IL-19, IL-20 (Truelove *et al.*, 2008), and Mdm2 p53 binding protein homolog (MDM2) (Yoon *et al.*, 2008), are associated with the risk of developing HCC. Consequently, specific SNPs can be used as genetic markers in patients with chronic liver diseases, including chronic hepatitis B (CHB) infections, liver cirrhosis (LC), and hepatitis B virus (HBV)-infected HCC.

HCC is one of the most common malignant tumors worldwide and causes about 1 million deaths each year (Parkin *et al.*, 2001; Marrero, 2006). The etiology of HCC seems to be multifactorial, and several events appear to be necessary for malignant transformation to occur. Hepatitis C virus (HCV) and HBV infections are important risk factors for chronic liver diseases (Collier & Sherman, 1998). CHB infection is the most common etiology of HCC in Asian countries. In particular, cirrhosis is present in about 70% to 80% of HCC cases (Velazquez *et al.*, 2003; Sy *et al.*, 2005). Moreover, HCC is a highly hypervascular tumor that is associated with a high faculty for vascular invasion (Sun & Tang, 2004). Because tumor angiogenesis plays a critical role in the development and progression of cancers, including HCC (Sun & Tang, 2004; Pang & Poon, 2007), angiogenic factors have been used not only for diagnosis and prognosis but also as predictors in cancer patients.

Erythropoietin-producing human hepatocellular carcinoma (Eph) receptors and ephrins are classified into the Ephrin-A or -B subfamily according to their structural homology and binding specificities. Eph receptors belong to the largest family of receptor tyrosine kinases, which contain an extracellular ephrin-binding domain and 2 fibronectin repeats, a transmembrane segment, and a cytoplasmic tyrosine kinase domain (Kalo & Pasquale, 1999). Eph receptor signaling is even more complex and involves the downstream activation of Src family kinases. Eph receptors interact with many downstream adaptors, including phosphotyrosine-binding adaptor proteins, such as Nck, Crk, Grb2, and Grb10 (Kullander & Klein, 2002). Importantly, Eph receptors and ephrins also regulate vascular development, tumorigenesis, and metastatic potential, as they are related to tumor growth and survival (Adams *et al*., 1999; Kojima *et al*., 2007; Kim *et al*., 2002). In addition, Eph/ephrin overexpression increases angiogenesis and tumor vasculature formation in many human cancers (Adams *et al*., 1999; Tang *et al*., 1999).

EphrinB ligands are transmembrane proteins that bind particularly to receptors of the EPHB subclass (Adams *et al*., 1999; Kojima *et al*., 2007). EphrinB1 is overexpressed in HCC, suggesting an important role of the Eph/ephrin system in hepatocarcinogenesis or tumor progression (Dodelet & Pasquale, 2000). The ephrinB1 ligand is known to bind to the EPHB1, EPHB2, and EPHB3 receptors, and the ectodomain of EPHB1 induces corneal angiogenesis (Brantley-Sieders & Chen, 2004). Recent reports suggest that a direct functional link between EphB/ephrinB signaling and integrins participates in angiogenesis (Brooks *et al*., 1994a).

Members of the integrin family of cell adhesion receptors play fundamental roles in angiogenesis. In particular, the integrin alpha v beta 3 is critical for angiogenesis and metastasis formation (Brooks *et al*., 1994b), (Brooks *et al*., 1994a; Kikkawa *et al*., 2002) and is significantly upregulated in activated endothelial cells during angiogenesis (Hood & Cheresh, 2002). Some alpha v integrins, particularly alpha v beta 3, are associated with tumor progression, including that of HCC (Lee *et al*., 2008). Despite these various findings, no study has addressed whether EPHB1 polymorphisms are associated with HCC. The aim of the present study was to determine whether EPHB1 polymorphisms are associated with susceptibility to CLD or HCC from acute hepatitis in a Korean population.

Methods

Subjects

This study included 86 HCC patients, 206 CLD patients, and 107 HBV clearance (CLE) patient controls. All subjects were of a genetically unrelated Korean population. All unrelated blood samples that were used in this study were obtained from the outpatient clinic of the Gastroenterology Department and from the Center for Health Promotion of Ajou University Hospital (Suwon, South Korea). This study was approved by the institutional review board.

Patients were classified into 3 groups-according to their HBV infection status, clinical data, and serological profile-as having CLE (spontaneous recovery or HBV eradication; n=107), CLD (including chronic hepatitis or liver cirrhosis, positivity for hepatitis B surface antigen [HBsAg (+)] for more than 6 months, and no hepatocellular carcinoma; n=206), or HCC (n=86). Patients who were positive for anti-HCV or anti-HIV antibodies were excluded. Serological tests were performed using commercially available assays for HBsAg and hepatitis B e antigen (HBeAg), antibodies against hepatitis B core (HBcAb) and hepatitis B e, liver function tests for aspartate aminotransferase (AST or SGOT), alanine aminotransferase (ALT or SGPT), alpha-fetoprotein (AFP), and bilirubin levels.

The 107 CLE control patients, who were HBsAg (−), HBcAb (+), and HBsAb (+), presented with acute hepatitis from which they subsequently recovered without any sequelae. Patients with chronic hepatitis showed elevated ALT ($\geq$2 times the upper limit of normal) at least 1 time during the follow-up period and were positive for HBV DNA, irrespective of HBeAg positivity. Patients with liver cirrhosis showed typical morphological findings on radiographic tests and corresponding laboratory features or evidence of portal hypertension. HCC was diagnosed if subjects fulfilled standard diagnostic criteria: either a serum AFP level $>$400 ng/ml in a patient who was known to have cirrhosis with hepatic masses as demonstrated on either ultrasonography or CT and diagnostic histology. The clinical characteristics of the study subjects are summarized in Table 1.

Sample preparation

Blood samples were stored at −80°C until use. Genomic DNA was purified using G-DEX blood genomic DNA (gDNA) purification kits (Intron Biotechnology Inc., SeungNam, Korea). The gDNA was quantified using the Picogreen dsDNA quantification reagent following a standard protocol (Molecular Probes, Eugene, OR, USA). The plates were read using a Victor TM 3 multilabel counter (PerkinElmer Inc. excitation 480 nm, emission 520 nm; PerkinElmer Inc., Waltham, MA, USA), and a standard curve for gDNA concentration was created using known concentrations of lambda DNA.

Table 1. Clinical characteristics of study subjects

Variable	CLE	CLD	HCC
No. of subjects	107	206	86
Gender (Male/Female)	80/27	158/48	69/17
Age (mean±SD)	46.04±8.40	42.86±9.62	53.88±10.58
HBsAb (Anti-HBs; positive rate, %)	100	0	0
HBsAg (positive rate, %)	0	100	100
Aspartate aminotransferase (U/L, mean±SD)	30.62±25.45	93.67±161.74	117.93±137.78
Alanine aminotransferase (U/L, mean±SD)	39.40±29.40	107.36±170.25	52.22±38.80
Albumin (g/dL, mean±SD)	4.40±0.26	3.96±0.61	3.39±0.69
Bilirubin (mg/dL, mean±SD)	0.90±0.35	1.71±2.88	2.63±4.23

Data are summarized as the mean±SD (standard deviation).
CLE, HBV clearance; CLD, Chronic Liver Disease; HCC, Hepatocellular Carcinoma

SNP selection and genotyping

EPHB1 was selected for further analysis based on the gene expression data that were obtained by comparing adjacent normal tissues and HCC samples with the GenePlorer TwinChip Human-8K human cDNA microarray (Digital Genomics, Seoul, Korea). Validated SNPs in the EPHB1 gene were selected from a public SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). As shown in Figure 1, there were 26 SNPs located in the 5 exons, 17 introns, and 4 5'-untranslated regions (UTRs) and flanking regions of EPHB1.

We selected intronic SNPs, for which genotyping was performed using the GoldenGate genotyping assay kit according to a standard protocol (Illumina Inc., San Diego, CA, USA). Briefly, 250 ng of gDNA was mixed with oligomers, and allele-specific extension was carried out by ramping the temperature from 70°C to 30°C over 16 h. Specific extension products were then used in polymerase chain reactions (PCRs) that consisted of 34 cycles of 35 s at 95°C, 35 s at 56°C, and 2 min at 72°C. PCR products were purified using 96-well filter plates (Millipore, Billerica, MA, USA). For hybridization, all samples were transferred to a 384-well microplate. The SAM chip and purified PCR products were hybridized at 60°C for 30 min and then at 45°C for 16 h. The SAM chip was then washed and imaged at a resolution of 0.8 mm using a BeadArray Reader (Illumina). Genotyping analysis was performed using BeadStudio software (Version 3.0.22, Illumina). Other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

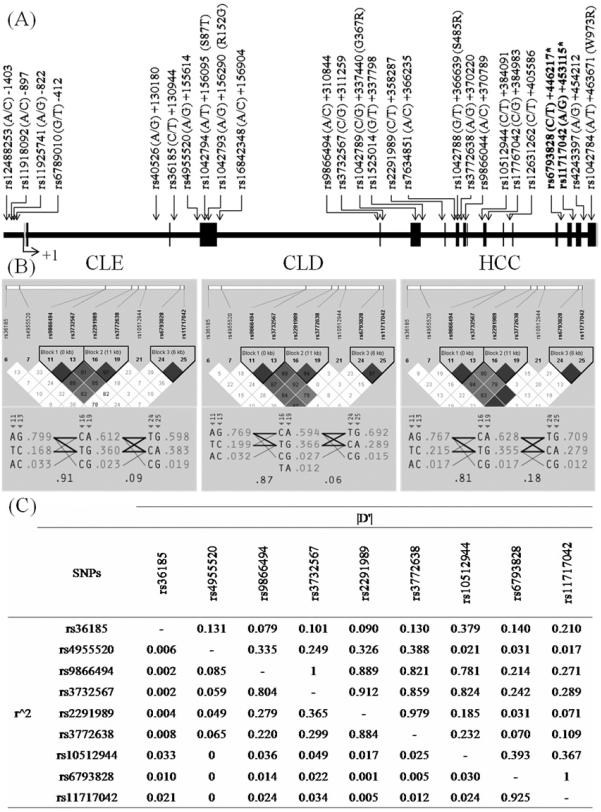


Fig. 1. Gene map of EPHB1 variations on chromosome 3q21-q23 and pair-wise linkage disequilibrium (LD) blocks. (A) Gene map and SNPs in EPHB1 on chromosome 3q21-q23. Exons are shown as black boxes, the 5'- and 3'-UTRs are represented by gray boxes, and the introns and 5'- and 3'-flanking regions are marked by lines. The first base of the translational start site is denoted as nucleotide +1. (B) Linkage disequilibriums (ID'I) among EPHB1 polymorphisms and haplotypes. An inverted triangle (▽) indicates haplotype-tagging SNPs. (C) LDs among EPHB1 SNPs in CLE.

Statistical analysis

The goodness of fit of the χ^2-test was used to compare the frequencies between the Korean (CLE) and Northeast Asian populations. Frequencies of the Northeast Asian population were from the Chinese and Japanese HapMap project data (http://hapmap.org). The χ^2-test was used to check the Hardy-Weinberg equilibrium. Four genetic models (allele, additive, dominant, and recessive) that were proposed by Lewis (Lewis 2002) were assumed for the association test between the CLD and HCC groups. Linkage disequilibrium (LD) blocks were confirmed by Haploview software (version

4.0; http//www.broad.mit.edu/mpg/haploview) using the confidence interval method. SNPs in the LD block included cases in which the minor allele frequency (MAF) was >0.1 and in Hardy-Weinberg equilibrium (p>0.05). Each individual haplotype was inferred using the SAS haplotype procedure. Multiple logistic regression models were used to calculate odds ratios (ORs), 95% confidence intervals, and corresponding p values and to control for age and gender as covariables (Oh *et al*., 2007). All statistical tests used SAS software (SAS Enterprise Guide 4.1; SAS Institute, Cary, NC, USA), and the significance level was set at 0.05.

Results

SNP genotyping, allele frequencies, and LD blocks

The EPHB1 gene is located on chromosome 3q21-q23 and comprises 16 exons. We examined the association between SNPs of the EPHB1 gene and HBV-infected liver diseases. BeadStudio software (version 2.0) was used for genotype analysis, and logistic regressions were used for association tests. We genotyped a total of 26 polymorphisms in EPHB1, including 3 in exonic regions, 4 in the 5'-flanking regions, and 17 in intronic regions. Of the 26 genotyped SNPs, 13 were monomorphic, 9 were polymorphic, and 4 were nonpolymorphic. The genotype distributions of all SNPs in the CLE and CLD groups and all except 2 SNPs (rs2291989 and rs3772638) in the HCC group were in Hardy-Weinberg equilibrium (data not shown). The locations and reference SNP allele of the identified polymorphic sites are shown in Fig. 1A.

The CLE control group served as a representative of the Korean population. Minor frequencies of the SNPs in the CLE group were compared with other Northeast Asian populations, including Chinese (HCB) and Japanese (JPT) populations, which were genotyped in the International HapMap Project. All frequencies, except those of 2 SNPs (rs36185 and rs4955520), were similar between the Korean and HCB+JPT populations (rs6793828; KOR 0.402 HCB+JPT 0.361 p=0.407, rs11717042; KOR 0.383 HCB+JPT 0.350 p=0.496, Table 2).

A comparison between the LD blocks of the CLE group of the Korean and Northeast Asian populations showed the similar pattern of LD blocks in EPHB1 (Figs. 1, 2). Moreover, the haplotype frequency patterns of HCB and JPT appeared to be the same as in our data (Table 2).

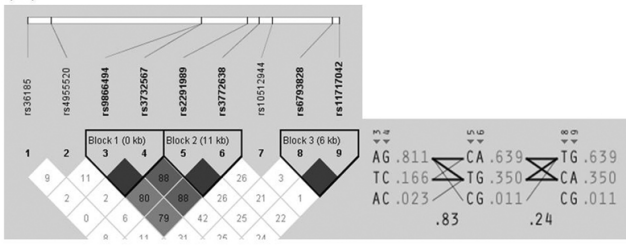
Table 2. Comparison of Koreans with Chinese and Japanese in MAF and haplotype frequency of EPHB1 polymorphism

SNP	Major	Minor	MAF		p value
			KOR	JPT+CHB	
rs36185	C	T	0.439	0.551	0.028
rs4955520	G	A	0.21	0.111	0.008
rs9866494	A	T	0.168	0.169	0.992
rs3732567	G	C	0.201	0.185	0.698
rs2291989	C	T	0.364	0.35	0.765
rs3772638	A	G	0.383	0.361	0.652
rs10512944	C	T	0.224	0.202	0.596
rs6793828	T	C	0.402	0.361	0.407
rs11717042	G	A	0.383	0.35	0.496

			Frequency		Percentage		p value
			Korean	HCB+JPT	Korean	HCB+JPT	
Block1	HT1	A-G	171	138	79.91	81.18	0.755
	HT2	T-C	36	28	16.82	16.47	0.927
Block2	HT3	C-A	131	115	61.21	63.89	0.585
	HT4	T-G	77	63	35.98	35	0.839
Block3	HT5	C-A	82	63	38.32	35	0.496
	HT6	T-G	128	115	59.81	63.89	0.407

Allele frequencies of North-East Asian groups were obtained from HapMap.
KOR, Korean; JPT, Japanese in Tokyo; HCB, Han Chinese in Beijing
p value, difference of minor allele frequency (MAF) was examined by chi-square test.
A, Major allele; B, Minor allele
A/A, Major homotype frequency; A/B, heterotype frequency; B/B, Minor homotype frequency

(A)



(B)

|D'|

	SNPs	rs36185	rs4955520	rs9866494	rs3732567	rs2291989	rs3772638	rs10512944	rs6793828	rs11717042
	rs36185		0.097	0.025	0.002	0.084	0.109	0.051	0.271	0.288
	rs4955520	0.001		0.113	0.028	0.066	0.114	0.257	0.049	0.104
	rs9866494	0	0		1.000	0.800	0.792	0.311	0.322	0.282
	rs3732567	0	0	0.850		0.888	0.884	0.423	0.253	0.225
r^2	rs2291989	0.003	0.001	0.242	0.327		1.000	0.268	0.250	0.246
	rs3772638	0.005	0.003	0.226	0.309	0.953		0.263	0.211	0.220
	rs10512944	0.001	0.002	0.005	0.010	0.033	0.031		0.033	0.012
	rs6793828	0.035	0	0.036	0.027	0.060	0.045	0		1.000
	rs11717042	0.038	0.001	0.029	0.022	0.061	0.046	0	0.953	

Fig. 2. Pair-wise linkage disequilibrium (LD) blocks of EPHB1 in Northeast Asian populations. (A) Linkage disequilibriums (|D'|) among EPHB1 polymorphisms and haplotypes. An inverted triangle (▽) indicates haplotype-tagging SNPs. (B) LDs among EPHB1 SNPs in Northeast Asian populations.

Association test of EPHB1 SNPs with HBV-infected liver diseases

With regard to genotypic association, we found that 2 SNPs (rs6793828 and rs11717042) were associated with HBV-infected liver diseases. The T allele of the SNP rs6793828 was associated with an increased risk of liver disease, with ORs of 0.610 (95% CI 0.40～0.94, p=0.024 in CLE vs HCC), 0.648 (95% CI 0.46～0.92, p=0.014 in CLE vs CLD), and 0.637 (95% CI 0.46～0.88, p=0.007 in CLE vs LD). The intronic SNP rs6793828 showed a higher risk with the homozygous variant TT genotype, with codominant ORs of 0.549 (95% CI 0.33～0.91, p=0.019 in CLE vs HCC), 0.636 (95% CI 0.44～0.92, p=0.015 in CLE vs CLD), and 0.615 (95% CI 0.44～0.87, p=0.006 in CLE vs LD); dominant ORs of 0.506 (95% CI 0.27～0.96, p=0.037 in CLE vs HCC) and 0.625 (95% CI 0.40～0.99, p=0.044 in CLE vs LD); and recessive ORs of 0.435 (95% CI 0.21～0.92, p=0.029 in CLE vs CLD) and 0.393 (95% CI 0.20～0.78, p=0.008 in CLE vs LD; Table 3).

The G allele of rs11717042 was associated with an increased risk of liver disease, with ORs of 0.623 (95% CI 0.40～0.96, p=0.032 in CLE vs HCC), 0.669 (95% CI 0.47～0.95, p=0.024 in CLE vs CLD), and 0.656 (95% CI 0.47～0.91, p=0.012 in CLE vs LD). Also, the intronic SNP rs11717042 showed an increased risk for the homozygous variant GG genotype, with codominant ORs of 0.573 (95% CI 0.35～0.95, p=0.030 in CLE vs HCC), 0.669 (95% CI 0.46～0.97, p=0.033 in CLE vs CLD), and 0.632 (95% CI 0.45～0.90, p=0.010 in CLE vs LD); and recessive ORs of 0.411 (95% CI 0.19～0.90, p=0.027 in CLE vs CLD) and 0.355 (95% CI 0.17～0.73, p=0.005 in CLE vs LD; Table 3).

EPHB1 haplotypes are associated with a risk for HBV-infected liver diseases

We found 3 LD blocks among the 9 polymorphic SNPs in EPHB1 that were constructed using the confidence interval method (Fig. 1B). All of the SNPs in the LD block were tagging SNPs, and each LD block included 2 SNPs. Only the intronic regions of EPHB1 had 6 markers (rs9866494, rs3732567, rs2291989, rs3772638, rs6793828, and rs11717042) with haplotypes that were displayed. Two common haplotypes (frequency ＞0.1) in each block were used for further analysis. The haplotype frequencies and association tests are summarized in Table 4. The low-frequency haplotypes were excluded from further analysis (data not shown). We identified a significant association between LD block3 and the risk for HBV-infected liver diseases, based on its 2 significant SNPs. Block3 had 3 haplotypes; in order of decreasing frequency, they were ht (C-A), ht (T-G), and ht (C-G). Except for the low-frequency haplotype (C-G), the frequencies with which each haplotype was present in the CLE, CLD, and HCC groups were estimated (Table 4).

Block3-ht5 (C-A) showed a protective effect, with codominant ORs of 1.744 (95% CI 1.06～2.88, p=0.030 in CLE vs HCC), 1.537 (95% CI 1.06～2.22, p=0.022 in CLE vs CLD), and 1.607 (95% CI 1.13～2.27, p=0.008 in CLE vs LD); dominant ORs of 2.434 (95% CI 1.11～5.34, p=0.027 in CLE vs HCC) and 2.817 (95% CI 1.37～5.81, p=0.005 in CLE vs LD); and a recessive OR of 1.520 (95% CI 0.97～2.39, p=0.070 in CLE vs LD). In contrast, block3-ht6 (T-G) showed a risk effect, with codominant ORs of 0.549 (95% CI 0.33～0.91, p=0.019 in CLE vs HCC), 0.654 (95% CI 0.45～0.94, p=0.023 in CLE vs CLD), and 0.624 (95% CI 0.44～0.88, p=0.007 in CLE vs LD); a dominant OR of 0.506 (95% CI 0.27～0.96, p=0.037 in CLE vs HCC); and recessive ORs of 0.435 (95% CI 0.21～0.92, p=0.029 in CLE vs CLD) and 0.393 (95% CI 0.20-0.78, p=0.008 in CLE vs LD; Table 4).

EPHB1 diplotypes are associated with a risk for HBV-infected liver diseases

EPHB1 diplotypes were reconstructed based on the significant haplotypes of LD block3, and their frequencies were estimated (Table 5). Association tests of low-fre-

Table 3. Genotype frequencies and associations between 13 EPHB1 polymorphisms with HBV-infected liver diseases

rsSNP	Geno-type	Frequency (%)			Model	CLE vs HCC		CLE vs CLD		CLE vs LD	
		CLE	CLD	HCC		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
rs36185	CC	33 (30.84)	69 (33.50)	24 (27.91)	Allele	1.163 (0.78～1.74)	0.462	0.952 (0.68～1.33)	0.772	1.010 (0.74～1.39)	0.949
C>T	CT	54 (50.47)	98 (47.57)	42 (48.84)	Codominant	1.195 (0.77～1.86)	0.431	0.948 (0.68～1.33)	0.755	1.014 (0.74～1.39)	0.932
intron	TT	20 (18.69)	39 (18.93)	20 (23.26)	Dominant	1.074 (0.54～2.12)	0.838	0.897 (0.54～1.49)	0.677	0.960 (0.59～1.55)	0.867
					Recessive	1.565 (0.72～3.39)	0.255	0.981 (0.53～1.80)	0.951	1.104 (0.63～1.94)	0.730
rs4955520	GG	67 (62.62)	149 (72.33)	52 (60.47)	Allele	1.065 (0.65～1.73)	0.800	0.691 (0.45～1.05)	0.087	0.795 (0.54～1.18)	0.251
G>A	GA	35 (32.71)	50 (24.27)	30 (34.88)	Codominant	1.131 (0.67～1.91)	0.645	0.697 (0.46～1.06)	0.092	0.796 (0.54～1.17)	0.247
intron	AA	5 (4.67)	7 (3.40)	4 (4.65)	Dominant	1.170 (0.62～2.21)	0.628	0.630 (0.38～1.04)	0.072	0.755 (0.48～1.20)	0.236
					Recessive	1.123 (0.27～4.59)	0.872	0.728 (0.22～2.39)	0.601	0.773 (0.26～2.29)	0.643
rs9866494	AA	73 (68.22)	133 (64.56)	52 (60.47)	Allele	1.355 (0.81～2.26)	0.243	1.229 (0.80～1.89)	0.350	1.265 (0.84～1.91)	0.262
A>T	AT	32 (29.91)	64 (31.07)	31 (36.05)	Codominant	1.485 (0.84～2.63)	0.175	1.250 (0.81～1.94)	0.319	1.270 (0.84～1.92)	0.260
intron	TT	2 (1.87)	9 (4.37)	3 (3.49)	Dominant	1.475 (0.77～2.83)	0.242	1.176 (0.71～1.95)	0.529	1.243 (0.78～1.99)	0.366
					Recessive	2.652 (0.42～16.62)	0.297	2.965 (0.59～14.80)	0.185	2.215 (0.49～10.08)	0.304
rs3732567	GG	69 (64.49)	120 (58.25)	50 (58.14)	Allele	1.205 (0.74～1.96)	0.453	1.192 (0.79～1.79)	0.396	1.196 (0.81～1.76)	0.364
G>C	GC	33 (30.84)	77 (37.38)	32 (37.21)	Codominant	1.394 (0.82～2.36)	0.215	1.195 (0.79～1.81)	0.399	1.209 (0.82～1.79)	0.344
intron	CC	5 (4.67)	9 (4.37)	4 (4.65)	Dominant	1.596 (0.83～3.06)	0.159	1.271 (0.78～2.08)	0.338	1.320 (0.83～2.09)	0.238
					Recessive	1.192 (0.30～4.69)	0.802	1.068 (0.34～3.40)	0.912	0.928 (0.32～2.68)	0.890
rs2291989	CC	45 (42.06)	80 (38.83)	31 (36.05)	Allele	0.958 (0.63～1.46)	0.842	1.062 (0.75～1.50)	0.729	1.031 (0.74～1.43)	0.854
C>T	CT	46 (42.99)	96 (46.60)	49 (56.98)	Codominant	1.085 (0.68～1.74)	0.736	1.038 (0.74～1.46)	0.831	1.038 (0.75～1.44)	0.827
intron	TT	16 (14.95)	30 (14.56)	6 (6.98)	Dominant	1.496 (0.79～2.85)	0.219	1.098 (0.68～1.78)	0.704	1.196 (0.76～1.88)	0.438
					Recessive	0.531 (0.19～1.50)	0.234	0.964 (0.49～1.88)	0.914	0.801 (0.42～1.51)	0.493
rs3772638	AA	41 (38.32)	77 (37.38)	29 (33.72)	Allele	0.954 (0.63～1.44)	0.824	1.043 (0.74～1.46)	0.808	1.016 (0.74～1.40)	0.922
A>G	AG	50 (46.73)	96 (46.60)	50 (58.14)	Codominant	1.058 (0.66～1.70)	0.818	1.009 (0.72～1.42)	0.960	1.021 (0.73～1.42)	0.903
intron	GG	16 (14.95)	33 (16.02)	7 (8.14)	Dominant	1.423 (0.74～2.72)	0.287	0.970 (0.59～1.58)	0.903	1.096 (0.69～1.73)	0.696
					Recessive	0.558 (0.20～1.54)	0.258	1.091 (0.56～2.11)	0.796	0.907 (0.48～1.70)	0.760
rs10512944	CC	65 (60.75)	121 (58.74)	53 (61.63)	Allele	0.916 (0.56～1.49)	0.724	1.022 (0.69～1.52)	0.913	0.990 (0.68～1.44)	0.959
C>T	CT	36 (33.64)	76 (36.89)	30 (34.88)	Codominant	1.042 (0.61～1.78)	0.882	1.058 (0.71～1.59)	0.786	0.986 (0.67～1.44)	0.943
intron	TT	6 (5.61)	9 (4.37)	3 (3.49)	Dominant	1.129 (0.60～2.13)	0.709	1.125 (0.69～1.82)	0.634	1.048 (0.67～1.65)	0.839
					Recessive	0.692 (0.15～3.28)	0.643	0.826 (0.28～2.43)	0.729	0.704 (0.26～1.93)	0.495
rs6793828	TT	38 (35.51)	96 (46.60)	41 (47.67)	Allele	0.610 (0.40～0.94)	**0.024**	0.648 (0.46～0.92)	**0.014**	0.637 (0.46～0.88)	**0.007**
T>C	TC	52 (48.60)	95 (46.12)	40 (46.51)	Codominant	0.549 (0.33～0.91)	**0.019**	0.636 (0.44～0.92)	**0.015**	0.615 (0.44～0.87)	**0.006**
intron	CC	17 (15.89)	15 (7.28)	5 (5.81)	Dominant	0.506 (0.27～0.96)	**0.037**	0.629 (0.39～1.02)	0.063	0.625 (0.40～0.99)	**0.044**
					Recessive	0.390 (0.13～1.18)	0.096	0.435 (0.21～0.92)	**0.029**	0.393 (0.20～0.78)	**0.008**
rs11717042	GG	41 (38.32)	98 (47.57)	42 (48.84)	Allele	0.623 (0.40～0.96)	**0.032**	0.669 (0.47～0.95)	**0.024**	0.656 (0.47～0.91)	**0.012**
G>A	GA	50 (46.73)	95 (46.12)	40 (46.51)	Codominant	0.573 (0.35～0.95)	**0.030**	0.669 (0.46～0.97)	**0.033**	0.632 (0.45～0.90)	**0.010**
intron	AA	16 (14.95)	13 (6.31)	4 (4.65)	Dominant	0.550 (0.29～1.04)	0.065	0.700 (0.43～1.13)	0.147	0.676 (0.43～1.06)	0.091
					Recessive	0.366 (0.11～1.20)	0.097	0.411 (0.19～0.90)	0.027	0.355 (0.17～0.73)	**0.005**

Logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding p-values for each SNP site and haplotype, controlling for age and sex as covariables using SAS. p-value of codominant, dominant, and recessive models also are given.

Model; Allele (A vs a), codominant (AA vs Aa vs aa), dominant (AA vs Aa plus aa), and recessive (AA plus Aa vs aa) models. CLE, HBV clearance; CLD, Chronic Liver Disease; HCC, Hepatocellular Carcinoma; LD, CLD+HCC; OR, odds ratio; CI, confidence interval

Bold indicates a case in which $p < 0.05$

quency diplotypes (<0.1) were skipped. Diplotypes of the 2 EPHB1 haplotypes (C-A and T-G) were associated with HBV-infected liver diseases. The (C-A/C-A) diplotype had a more protective effect against HBV-infected liver diseases, including CLD and HCC, than the (T-G/T-G) diplotype. In addition, the (T-G/T-G) diplotype showed a greater relative risk effect for HBV-infected liver diseases than the (C-A/C-A) diplotype (Table 5).

Discussion

In this study, we showed that the genetic polymor-

Table 4. Analysis of haplotypes in EPHB1

Block	Haplo-type	Type	Frequency			Model	CLE vs HCC		CLE vs CLD		CLE vs LD	
			CLE (%)	CLD (%)	HCC (%)		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Block1	HT1	+/+	69 (64.49)	120 (58.25)	50 (58.14)	Codominant	1.394 (0.82～2.36)	0.215	1.195 (0.79～1.81)	0.399	1.209 (0.82～1.79)	0.344
	A-G	+/-	33 (30.84)	77 (37.38)	32 (37.21)	Dominant	1.596 (0.83～3.06)	0.159	1.271 (0.78～2.08)	0.338	1.320 (0.83～2.09)	0.238
		-/-	5 (4.67)	9 (4.37)	4 (4.65)	Recessive	1.192 (0.30～4.69)	0.802	1.068 (0.34～3.40)	0.912	0.928 (0.32～2.68)	0.890
	HT2	+/+	2 (1.87)	9 (4.37)	3 (3.49)	Codominant	0.673 (0.38～1.19)	0.175	0.800 (0.52～1.24)	0.319	0.788 (0.52～1.19)	0.260
	T-C	+/-	32 (29.91)	64 (31.07)	31 (36.05)	Dominant	0.377 (0.06～2.36)	0.297	0.337 (0.07～1.68)	0.185	0.451 (0.10～2.05)	0.304
		-/-	73 (68.22)	133 (64.56)	52 (60.47)	Recessive	0.678 (0.35～1.30)	0.242	0.850 (0.51～1.41)	0.529	0.805 (0.50～1.29)	0.366
Block2	HT3	+/+	41 (38.32)	73 (35.44)	29 (33.72)	Codominant	1.033 (0.64～1.65)	0.893	1.037 (0.74～1.46)	0.834	1.038 (0.75～1.44)	0.824
	C-A	+/-	49 (45.79)	99 (48.06)	50 (58.14)	Dominant	1.423 (0.74～2.72)	0.287	1.052 (0.64～1.72)	0.840	1.164 (0.74～1.84)	0.516
		-/-	17 (15.89)	34 (16.50)	7 (8.14)	Recessive	0.518 (0.19～1.41)	0.198	1.045 (0.55～1.99)	0.894	0.867 (0.47～1.60)	0.650
	HT4	+/+	15 (14.02)	29 (14.08)	6 (6.98)	Codominant	0.899 (0.56～1.45)	0.663	0.991 (0.70～1.40)	0.957	0.980 (0.70～1.37)	0.906
	T-G	+/-	47 (43.93)	93 (45.15)	49 (56.98)	Dominant	1.738 (0.60～4.99)	0.305	0.993 (0.50～1.97)	0.984	1.194 (0.62～2.29)	0.593
		-/-	45 (42.06)	84 (40.78)	31 (36.05)	Recessive	0.668 (0.35～1.27)	0.219	0.985 (0.61～1.59)	0.951	0.887 (0.56～1.39)	0.601
Block3	HT5	+/+	16 (14.95)	13 (6.31)	4 (4.65)	Codominant	1.744 (1.06～2.88)	**0.030**	1.537 (1.06～2.22)	**0.022**	1.607 (1.13～2.27)	**0.008**
	C-A	+/-	50 (46.73)	93 (45.15)	40 (46.51)	Dominant	2.734 (0.83～8.97)	0.097	2.434 (1.11～5.34)	**0.027**	2.817 (1.37～5.81)	**0.005**
		-/-	41 (38.32)	100 (48.54)	42 (48.84)	Recessive	1.817 (0.96～3.43)	0.065	1.505 (0.93～2.44)	0.096	1.520 (0.97～2.39)	**0.070**
	HT6	+/+	38 (35.51)	94 (45.63)	41 (47.67)	Codominant	0.549 (0.33～0.91)	**0.019**	0.654 (0.45～0.94)	**0.023**	0.624 (0.44～0.88)	**0.007**
	T-G	+/-	52 (48.60)	97 (47.09)	40 (46.51)	Dominant	0.506 (0.27～0.96)	**0.037**	0.663 (0.41～1.08)	0.099	0.643 (0.41～1.02)	0.059
		-/-	17 (15.89)	15 (7.28)	5 (5.81)	Recessive	0.390 (0.13～1.18)	0.096	0.435 (0.21～0.92)	**0.029**	0.393 (0.20～0.78)	**0.008**

EPHB1 haplotypes constructed from 6 SNPs and their frequencies in the Korean population (n=399). Genotype distributions in all loci were in Hardy-Weinberg equilibrium ($p > 0.05$). CLE, HBV clearance; CLD, Chronic Liver Disease; HCC, Hepatocellular Carcinoma; LD, CLD+HCC; OR, odds ratio; CI, confidence interval

Table 5. Analysis of diplotypes in LD block3 of EPHB1

CLE vs CLD				OR		
Diplotype	CLE (%)	CLD (%)	p value	C-A/C-A	C-A/T-G	T-G/T-G
C-A/C-A	16 (14.95)	13 (6.31)		-	0.47	**0.353**
C-A/T-G	49 (45.79)	91 (44.17)		0.073	-	0.752
T-G/T-G	38 (35.51)	94 (45.63)		**0.015**	0.282	-
CLE vs HCC				OR		
Diplotype	CLE (%)	HCC (%)	p value	C-A/C-A	C-A/T-G	T-G/T-G
C-A/C-A	16 (14.95)	4 (4.65)		-	0.459	**0.27**
C-A/T-G	49 (45.79)	39 (45.35)		0.217	-	0.589
T-G/T-G	38 (35.51)	41 (47.67)		**0.039**	0.123	-
CLE vs LD				OR		
Diplotype	CLE (%)	LD (%)	p value	C-A/C-A	C-A/T-G	T-G/T-G
C-A/C-A	16 (14.95)	17 (5.82)		-	**0.404**	**0.302**
C-A/T-G	49 (45.79)	130 (44.52)		**0.019**	-	0.748
T-G/T-G	38 (35.51)	135 (46.23)		**0.002**	0.243	-

EPHB1 diplotypes constructed from 2 haplotypes and their frequencies in the Korean population (n=399). CLE, HBV clearance; CLD, Chronic Liver Disease; HCC, Hepatocellular Carcinoma; LD, CLD+HCC; OR, odds ratio; CI, confidence interval Bold indicates a case in which $p < 0.05$

phisms and haplotypes of EPHB1 are associated with a risk for HBV-infected liver diseases in a Korean population. We identified 2 significant SNPs (rs6793828 and rs11717042) in LD block3 of EPHB1 and correlated them with HBV-infected liver diseases. The SNPs rs6793828 and rs11717042 were in strong LD and strongly affected the risk for HBV-associated liver diseases as haplotypes. Moreover, the pattern of EPHB1 LD blocks in Korean population is similar to those in other Northeast Asian populations. Our study indicates that HBV infection is similar among Northeast Asian groups, including CHB and JPT populations (The Korean HapMap Project Website (Kim *et al.*, 2008)).

The 2 SNPs in EPHB1 that are associated with HBV-infected liver diseases, including CLD and HCC, are likely to be true risk factors due to their sufficient haplotype and diplotype effects (Table 4, 5). Carriers of the ht5 (C-A) haplotype-containing variant alleles of these SNPs displayed protective effects (Table 4). Furthermore, a significantly decreased risk also was observed for the ht5/ht5 (C-A/C-A) diplotype. In contrast, ht6 (T-G) and ht5/ht6 (C-A/T-G) displayed significant risk effects. Either of the 2 SNPs (rs6793828 and rs11717042) of EPHB1 may be candidates for a functional polymorphism that may affect gene expression and cancer aggressiveness.

Eph receptor tyrosine kinases initially were considered to be putative oncogenes, based on their overexpression in various human cancers, including HCC (Clifford *et al.*, 2008; Chang *et al.*, 2008; Oshima *et al.*, 2008; Sawai *et al.*, 2003). EPHB1 SNPs have been shown to be associated with cancers, such as esophageal squamous cell carcinoma (ESCC) (Ng *et al.*, 2008; Hu *et al.*, 2005; Lesnick *et al.*, 2007), and previous studies identified the association of a significant SNP, rs6793828, with Parkinson disease (Lesnick *et al.*, 2007). Eph receptor "forward signaling" depends on the tyrosine kinase domain, which mediates adaptor protein phosphorylation and Eph receptor association with various proteins (Pasquale, 2008; Himanen & Nikolov, 2003a). Other signaling proteins interact with Eph receptors and cause a variety of cellular effects, such as cell adhesion and migration (Castano *et al.*, 2008).

EPHB1 is a member of the Eph receptor family that mediates vascular development. Recently, the activation of endogenous EPHB1 receptors on renal microvascular endothelial cells by ephrinB1 was shown to induce tubule formation. EphrinB1 expression promotes tumor growth by initiating tumor angiogenesis in HCC (Valentini *et al.*, 2003). Interestingly, we found that the haplotypes were located in the Eph tyrosine kinase domain of EPHB1. The phosphorylated tyrosines of EPHB1 interact with the Src homology 2 domains of signaling molecules that are associated with HCC (Klein & Schneider, 1997; Yoon *et al.*, 2001; Stein *et al.*, 1998), such as Src, growth factor receptor-bound protein 2 (Grb2), and Nck (Pasquale, 2008; Himanen & Nikolov, 2003a; Himanen & Nikolov, 2003b). Thus, the LD region that contains ht6 (T-G), including some causative SNPs, may have synergistic and antagonistic effects on various downstream signaling proteins.

We suggest that ht6 (T-G) of EPHB1 is overrepresented in HBV-infected liver disease patients and that it potentially facilitates integrin alpha v beta 3-mediated increases in attachment of endothelial cells and activated integrin alpha v beta 3-regulated angiogenesis in HCC. These results provide the first evidence that functional ht6 (T-G) of EPHB1 is a genetic susceptibility factor for the development of CLD and HCC in a Korean population.

Angiogenesis plays an important role in the liver, and its role in HCC has been studied. Recently, it was reported that HCC and chronic HBV infection are associated with alpha v integrins (Lee *et al.*, 2008). In particular, the beta 3 subset has been shown to mediate endothelial cell attachment (Huynh-Do *et al.*, 1999). The integrin alpha v beta 3 is expressed particularly by endothelial cells that are involved in angiogenesis, as its expression clearly is increased in proliferating vascular endothelial cells (Brooks *et al.*, 1994a; Drake *et al.*, 1995). Moreover, EPHB1 must be signaling-competent in order to mediate ephrinB1-induced attachment responses. The ephrinB1-induced increase in cell attachment suggests 'inside-out' activation of alpha v beta 3 in endothelial cells (Huynh-Do *et al.*, 1999). Eph receptors regulate cell attachment to the extracellular matrix by modulating integrin activity.

Taken together, our results show that EPHB1 SNPs, haplotypes, and diplotypes are relevant susceptibility markers for HBV-infected liver diseases. In previous studies, EPHB1 signaling has been shown to be associated with alpha v beta 3. SNPs and haplotypes of alpha v integrins are associated with HBV-infected HCC (Lee *et al.*, 2008). Further studies are needed to demonstrate by gene-gene interactions that SNPs and haplotypes of EPHB1 and integrin alpha v are associated with HBV-infected liver diseases.

Acknowledgments

This work was supported by grants from the Ministry of Health and Welfare, Republic of Korea (A010383), and from the Health 21 R&D Project of the Ministry of Health and Welfare, Republic of Korea (A050558).

References

Adams, R.H., Wilkinson, G.A., Weiss, C., Diella, F., Gale, N.W., Deutsch, U., Risau, W., and Klein, R. (1999). Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. *Genes. Dev.* 13, 295-306.

Brantley-Sieders, D.M., and Chen, J. (2004). Eph receptor tyrosine kinases in angiogenesis: from development to disease. *Angiogenesis* 7, 17-28.

Brooks, P.C., Clark, R.A., and Cheresh, D.A. (1994a). Requirement of vascular integrin alpha v beta 3 for angiogenesis. *Science* 264, 569-571.

Brooks, P.C., Montgomery, A.M., Rosenfeld, M., Reisfeld, R.A., Hu, T., Klier, G., and Cheresh, D.A. (1994b). Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 79, 1157-1164.

Calin, G.A., Trapasso, F., Shimizu, M., *et al.* (2005). Familial cancer associated with a polymorphism in ARLTS1. *N. Engl. J. Med.* 352, 1667-1676.

Castano, J., Davalos, V., Schwartz, S., Jr., and Arango, D. (2008). EPH receptors in cancer. *Histol. Histopathol.* 23, 1011-1023.

Chang, Q., Jorgensen, C., Pawson, T., and Hedley, D.W. (2008). Effects of dasatinib on EphA2 receptor tyrosine kinase activity and downstream signalling in pancreatic cancer. *Br. J. Cancer* 99, 1074-1082.

Clifford, N., Smith, L.M., Powell, J., Gattenlohner, S., Marx, A., and O'Connor, R. (2008). The EphA3 receptor is expressed in a subset of rhabdomyosarcoma cell lines and suppresses cell adhesion and migration. *J. Cell. Biochem.* (in press).

Collier, J., and Sherman, M. (1998). Screening for hepatocellular carcinoma. *Hepatology* 27, 273-278.

Dodelet, V.C., and Pasquale, E.B. (2000). Eph receptors and ephrin ligands: embryogenesis to tumorigenesis. *Oncogene* 19, 5614-5619.

Drake, C.J., Cheresh, D.A., and Little, C.D. (1995). An antagonist of integrin alpha v beta 3 prevents maturation of blood vessels during embryonic neovascularization. *J. Cell. Sci.* 108(Pt 7), 2655-2661.

Dutt, A., and Beroukhim, R. (2007). Single nucleotide polymorphism array analysis of cancer. *Curr. Opin. Oncol.* 19, 43-49.

Fu, X., Wang, Q., Chen, X., Huang, X., Cao, L., Tan, H., Li, W., Zhang, L., Bi, J., Su, Q., and Chen, L. (2008). Expression patterns and polymorphisms of PTCH in Chinese hepatocellular carcinoma patients. *Exp. Mol. Pathol.* 84, 195-199.

Himanen, J.P., and Nikolov, D.B. (2003a). Eph receptors and ephrins. *Int. J. Biochem. Cell. Biol.* 35, 130-134.

Himanen, J.P., and Nikolov, D.B. (2003b). Eph signaling: a structural view. *Trends. Neurosci.* 26, 46-51.

Hood, J.D., and Cheresh, D.A. (2002). Role of integrins in cell invasion and migration. *Nat. Rev. Cancer* 2, 91-100.

Hu, N., Wang, C., Hu, Y., Yang, H.H., Giffen, C., Tang, Z.Z., Han, X.Y., Goldstein, A.M., Emmert-Buck, M.R., Buetow, K.H., Taylor, P.R., and Lee, M.P. (2005). Genome-wide association study in esophageal cancer using GeneChip mapping 10K array. *Cancer Res.* 65, 2542-2546.

Huynh-Do, U., Stein, E., Lane, A.A., Liu, H., Cerretti, D.P., and Daniel, T.O. (1999). Surface densities of ephrin-B1 determine EphB1-coupled activation of cell attachment through alphavbeta3 and alpha5beta1 integrins. *EMBO. J.* 18, 2165-2173.

Kalo, M.S., and Pasquale, E.B. (1999). Signal transfer by Eph receptors. *Cell Tissue Res.* 298, 1-9.

Kikkawa, H., Kaihou, M., Horaguchi, N., Uchida, T., Imafuku, H., Takiguchi, A., Yamazaki, Y., Koike, C., Kuruto, R., Kakiuchi, T., Tsukada, H., Takada, Y., Matsuura, N., and Oku, N. (2002). Role of integrin alpha(v)beta3 in the early phase of liver metastasis: PET and IVM analyses. *Clin. Exp. Metastasis* 19, 717-725.

Kim, I., Ryu, Y.S., Kwak, H.J., Ahn, S.Y., Oh, J.L., Yancopoulos, G.D., Gale, N.W., and Koh, G.Y. (2002). EphB ligand, ephrinB2, suppresses the VEGF- and angiopoietin 1-induced Ras/mitogen-activated protein kinase pathway in venous endothelial cells. *FASEB. J.* 16, 1126-1128.

Kim, Y.U., Kim, S.H., Jin, H., Park, Y.K., Ji, M.H., and Kim, Y.J. (2008). The Korean HapMap Project Website. *Genomics & Informatics* 6, 91-94.

Kiyohara, C., and Yoshimasu, K. (2007). Genetic polymorphisms in the nucleotide excision repair pathway and lung cancer risk: a meta-analysis. *Int. J. Med. Sci.* 4, 59-71.

Klein, N.P., and Schneider, R.J. (1997). Activation of Src family kinases by hepatitis B virus HBx protein and coupled signaling to Ras. *Mol. Cell. Biol.* 17, 6427-6436.

Kojima, T., Chang, J.H., and Azar, D.T. (2007). Proangiogenic role of ephrinB1/EphB1 in basic fibroblast growth factor-induced corneal angiogenesis. *Am. J. Pathol.* 170, 764-773.

Kullander, K., and Klein, R. (2002). Mechanisms and functions of Eph and ephrin signalling. *Nat. Rev. Mol. Cell. Biol.* 3, 475-486.

Lee, S.K., Kim, M.H., Cheong, J.Y., Cho, S.W., Yang, S.J., and Kwack, K. (2008). Integrin alpha V polymorphisms and haplotypes in a Korean population are associated with susceptibility to chronic hepatitis and hepatocellular carcinoma. *Liver. Int.* (in press).

Lesnick, T.G., Papapetropoulos, S., Mash, D.C., Ffrench-Mullen, J., Shehadeh, L., de Andrade, M., Henley, J.R., Rocca, W.A., Ahlskog, J.E., and Maraganore, D.M. (2007). A genomic pathway approach to a complex disease: axon guidance and Parkinson disease. *PLoS Genet.* 3, e98.

Lewis, C.M. (2002). Genetic association studies: design, analysis and interpretation. *Brief Bioinform.* 3, 146-153.

Marrero, J.A. (2006). Hepatocellular carcinoma. *Curr. Opin. Gastroenterol.* 22, 248-253.

Ng, D., Hu, N., Hu, Y., Wang, C., Giffen, C., Tang, Z.Z., Han, X.Y., Yang, H.H., Lee, M.P., Goldstein, A.M., and Taylor, P.R. (2008). Replication of a genome-wide case-control study of esophageal squamous cell carcinoma.

Int. J. Cancer. 123, 1610-1615.
Oh, S.H., and Park, T.S. (2007). Regression Models for Haplotype-Based Association Studies. *Genomics & Informatics* 5, 19-23.
Oshima, T., Akaike, M., Yoshihara, K., Shiozawa, M., Yamamoto, N., Sato, T., Akihito, N., Nagano, Y., Fujii, S., Kunisaki, C., Wada, N., Rino, Y., Tanaka, K., Masuda, M., and Imada, T. (2008). Overexpression of EphA4 gene and reduced expression of EphB2 gene correlates with liver metastasis in colorectal cancer. *Int. J. Oncol.* 33, 573-577.
Pang, R.W., and Poon, R.T. (2007). From molecular biology to targeted therapies for hepatocellular carcinoma: the future is now. *Oncology* 72 Suppl 1, 30-44.
Parkin, D.M., Bray, F., Ferlay, J., and Pisani, P. (2001). Estimating the world cancer burden: Globocan 2000. *Int. J. Cancer.* 94, 153-156.
Pasquale, E.B. (2008). Eph-ephrin bidirectional signaling in physiology and disease. *Cell* 133, 38-52.
Sawai, Y., Tamura, S., Fukui, K., Ito, N., Imanaka, K., Saeki, A., Sakuda, S., Kiso, S., and Matsuzawa, Y. (2003). Expression of ephrin-B1 in hepatocellular carcinoma: possible involvement in neovascularization. *J. Hepatol.* 39, 991-996.
Stein, E., Huynh-Do, U., Lane, A.A., Cerretti, D.P., and Daniel, T.O. (1998). Nck recruitment to Eph receptor, EphB1/ELK, couples ligand activation to c-Jun kinase. *J. Biol. Chem.* 273, 1303-1308.
Storey, A., Thomas, M., Kalita, A., Harwood, C., Gardiol, D., Mantovani, F., Breuer, J., Leigh, I.M., Matlashewski, G., and Banks, L. (1998). Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. *Nature* 393, 229-234.
Sun, H.C., and Tang, Z.Y. (2004). Angiogenesis in hepatocellular carcinoma: the retrospectives and perspectives. *J. Cancer. Res. Clin. Oncol.* 130, 307-319.
Sy, S.M., Wong, N., Lai, P.B., To, K.F., and Johnson, P.J. (2005). Regional over-representations on chromosomes 1q, 3q and 7q in the progression of hepatitis B virus-related hepatocellular carcinoma. *Mod. Pathol.* 18, 686-692.
Tang, X.X., Brodeur, G.M., Campling, B.G., and Ikegaki, N. (1999). Coexpression of transcripts encoding EPHB receptor protein tyrosine kinases and their ephrin-B ligands in human small cell lung carcinoma. *Clin. Cancer Res.* 5, 455-460.
Truelove, A.L., Oleksyk, T.K., Shrestha, S., Thio, C.L., Goedert, J.J., Donfield, S.M., Kirk, G.D., Thomas, D.L., O'Brien, S.J., and Smith, M.W. (2008). Evaluation of IL10, IL19 and IL20 gene polymorphisms and chronic hepatitis B infection outcome. *Int. J. Immunogenet.* 35, 255-264.
Valentini, F.A., Nelson, P.P., and Besson, G.R. (2003). Detrusor activity and motor neurons firing during micturition: analysis by means of modeling of urodynamic tracings. *Ann. Readapt. Med. Phys.* 46, 594-600.
Velazquez, R.F., Rodriguez, M., Navascues, C.A., Linares, A., Perez, R., Sotorrios, N.G., Martinez, I., and Rodrigo, L. (2003). Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. *Hepatology* 37, 520-527.
Xiong, X.D., Fang, J.H., Qiu, F.E., Zhao, J., Cheng, J., Yuan, Y., Li, S.P., and Zhuang, S.M. (2008). A novel functional polymorphism in the Cdc6 promoter is associated with the risk for hepatocellular carcinoma. *Mutat. Res.* 643, 70-74.
Yoon, S.Y., Jeong, M.J., Yoo, J., Lee, K.I., Kwon, B.M., Lim, D.S., Lee, C.E., Park, Y.M., and Han, M.Y. (2001). Grb2 dominantly associates with dynamin II in human hepatocellular carcinoma HepG2 cells. *J. Cell. Biochem.* 84, 150-155.
Yoon, Y.J., Chang, H.Y., Ahn, S.H., Kim, J.K., Park, Y.K., Kang, D.R., Park, J.Y., Myoung, S.M., Kim do, Y., Chon, C.Y., and Han, K.H. (2008). MDM2 and p53 polymorphisms are associated with the development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Carcinogenesis* 29, 1192-1196.
Zeng, Z.Y., Xiong, W., Zhou, Y.H., Li, X.L., and Li, G.Y. (2006). Advances in high-density whole genome-wide single nucleotide polymorphism array in cancer research. *Ai Zheng* 25, 1454-1458.