GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 17, Number 8, 2013

Pp. 595–600
DOI: 10.1089/gtmb.2013.0031

Association of Epidermal Growth Factor and Epidermal Growth Factor Receptor Polymorphisms with the Risk of Hepatitis B Virus-Related Hepatocellular Carcinoma in the Population of North China

Jia Wu,[1] Wei Zhang,[2] Aiqiang Xu,[3] Li Zhang,[3] Tao Yan,[4] Zhuo Li,[5] Xiaopan Wu,[1] Xilin Zhu,[1] Juan Ma,[1] Ke Li,[4] Hui Li,[6] and Ying Liu[1]

Background: Hepatocellular carcinoma (HCC) is a common solid malignant tumor occurring worldwide that leads to the third largest cause of death compared to other cancers. Genetic and environmental factors are involved in the pathogenesis of HCC. Epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) can stimulate the proliferation of epidermal and epithelial cells. The EGF signal pathway has a relationship with the growth of the embryo, tissue repairing, and tumorigenesis. *Methods:* In this study, 416 patients with hepatitis B virus infection (HBV)-related HCC and 645 individuals who had never been infected with HBV of the Chinese Han population were enrolled. Eight single-nucleotide polymorphisms (SNPs), whose minor allele frequency >20% in the *EGF* and *EGFR* genes, were genotyped to examine their associations with hepatocarcinogenesis. Genotyping experiments were carried out using TaqMan. *Results:* There were significant differences in genotype distributions ($p=0.005$) and allele frequencies ($p=0.001$, odds ratio [OR]=1.43, 95% confidence interval [CI]=1.15–1.79) of rs11569017 in the *EGF* gene between the HCC and control groups. After binary logistic regression to determine independent factors for susceptibility to HCC under an additive model, rs11569017 was still independently associated with the susceptibility to HCC ($p=0.021$, OR=1.48, 95% CI=1.06–2.07), but no significant differences in other SNPs were found. Additionally, the haplotype T-G constructed by rs11569017 and rs4444903 of the *EGF* gene might increase the risk of HBV-related HCC ($p=0.002$, OR=1.44, 95% CI=1.15–1.82). *Conclusion:* The rs11569017 T allele was associated with susceptibility to HBV-related HCC.

Introduction

Hepatocellular carcinoma (HCC) is a common solid malignant tumor occurring worldwide and representing the third leading cause of death compared with other cancers. Liver cancer predominates among males in developing regions (Taylor *et al.*, 2008). HCC is a kind of cancer with multiple etiologies (Anzola, 2004), chronic infection with hepatitis B, excessive alcohol consumption, and many etiological factors have been related with HCC development (Whittaker *et al.*, 2010). The genetic factors play an important role in the occurrence of HCC (Zhang *et al.*, 2010). However, its molecular mechanism is not yet clear.

In recent years, a number of important signaling pathways in HCC have been studied systematically. These pathways regulate physiological processes such as the growth and differentiation of tumor cells, the regeneration of blood vessels, and the migration of tumor cells (Zender *et al.*, 2010). Studies have shown that the signal pathway of the epidermal growth factor (EGF) plays an important role in the occurrence of liver cancer. The EGF can stimulate the proliferation of epidermal and epithelial cells, which have a strong relationship with the growth of the embryo, tissue repair, regeneration, and tumorigenesis (Fisher and Lakshmanan, 1990; Limaye *et al.*, 2008). The transient profile of EGF RNA accumulation suggests that an increase in EGF levels may catalyze a cascade of

[1]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, School of Basic Medicine, Peking Union Medical College, Beijing, People's Republic of China.
[2]Beijing Center for Disease Control and Prevention, Beijing, People's Republic of China.
[3]Shandong Center for Disease Control and Prevention, Jinan, People's Republic of China.
[4]Liver Failure Treatment and Research Center, The 302 Hospital of the PLA, Beijing, People's Republic of China.
[5]Department of Infectious Disease, Affiliated Youan Hospital, Capital University of Medical Sciences, Beijing, People's Republic of China.
[6]Department of Epidemiology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, School of Basic Medicine, Peking Union Medical College, Beijing, People's Republic of China.

events preceding the first wave of hepatic DNA replication in hepatocytes isolated by collagenase perfusion (Mullhaupt *et al*., 1994). The EGF activates the epidermal growth factor receptor (EGFR) as a ligand with biological effect through signal transduction (Villanueva *et al*., 2007).

The EGFR is a kind of membrane receptor, which overexpresses in many kinds of malignant tumors such as breast cancer (Nakajima *et al*., 2012), lung cancer (Han *et al*., 2011), ovarian cancer, cervical cancer, esophageal cancer, prostate cancer, and liver cancer (Han and Lo, 2012). The EGFR accelerates proliferation and metastasis of tumor cells along with promoting tumor angiogenesis and hindering tumor apoptosis.

The EGFR belongs to the receptor tyrosine kinase family and widely distributes on epithelial cells. Ligands integrating with the EGFR form a homodimer, after a process of phosphorylation, the system of ras/raf/MAPK cascade is activated, then the dimer turns into the affinity sites of downstream signal transduction to generate high-affinity interactions with the signal transduction molecules involved in mitosis (Kenny, 2007).

Currently, a number of molecular genetic studies have shown that the polymorphisms of the *EGFR* and its ligand genes are related to lung cancer (Dong *et al*., 2010), kidney cancer (Zhu *et al*., 2010), breast cancer (Choura *et al*., 2010), and other cancers. The association studies between the *EGF* gene and HCC were mainly concentrated in the 61*A/G polymorphism (rs4444903), however, the results are inconsistent (Li *et al*., 2009; Qi *et al*., 2009; Zhong *et al*., 2012). In this study, we aim to investigate the association between polymorphisms in the *EGFR* gene and its ligand *EGF* gene and hepatitis B virus (HBV)-related HCC in the Chinese Han population.

Materials and Methods

Subjects

The subjects enrolled in the study included a total of 1061 northern Chinese Han participants, who were divided into two groups: the case group consisted of 416 patients (352 men/64 women, mean age 54.5 ± 10.36 years) with HBV-related HCC; the control group consisted of 645 individuals who had never been infected with HBV (522 men/123 women, mean age 39.3 ± 6.90 years), in whom all HBV serum markers were negative (the data for detailed patient profile are shown in Supplementary Table S2; Supplementary Data are available online at www.liebertpub.com/gtmb). They were recruited from 302 Hospital of the People's Liberation Army, Beijing Youan Hospital, Beijing Center for Disease Control and Prevention (CDC), and Shandong CDC between November 2001 and October 2010. All participants with HBV-related HCC were pathologically HCC patients, who were also on the basis of clinical evidence obtained from liver function tests and serum immunologic marker screening, proved not to have other cancers. They were confirmed by liver ultrasonography and/or computed tomography as well (Xin *et al*., 2012).

The subjects were excluded if (1) they had past or present infection with other hepatitis viruses or hepatitis not caused by HBV; (2) they were not of Han ethnicity; and (3) they had alcoholic liver disease, autoimmune liver disease, or metabolic liver disease. The study was performed in accordance with the guidelines of the Helsinki Declaration after informed consent had been obtained from all the participants. It was approved by the ethics committee of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

Single-nucleotide polymorphism selection and genotyping

Genomic DNA was extracted from peripheral blood by using a method of salting-out (Miller *et al*., 1988). A strategy of functional single-nucleotide polymorphisms (SNPs) selecting was applied. The entire gene region from the 5′untranslated region (5′ UTR) to 3′UTR was selected using the HapMap database (HapMap Data Rel 24/phase II Nov 08, on NCBI B36 assembly, dbSNP b126) with minor allele frequencies (MAF) > 0.20. The strategy we chose could make sure that all SNPs selected have no linkage disequilibrium (LD). In the *EGF*, the SNP rs11569017 located in exon15 and rs4444907 located in 5′UTR were selected, and rs11569017 as a TagSNP represented another 14 SNPs. In the *EGFR*, the six SNPs (rs2072454, rs2227983, rs17337023, rs1050171, rs2293347, and rs884225) were selected for the present study (shown in Table 1). Among all the SNPs in the *EGFR*, rs2072454, rs2227983, rs17337023, rs1050171, and rs2293347 were in exons, and rs884225 was in the 3′UTR.

The genotyping was conducted using TaqMan, with probes synthesized by Sangon BioTech Co., Ltd. The sequences of the polymerase chain reaction (PCR) primers and the probes were listed in Supplementary Table S1. A volume of 10 μL amplification reactions was carried out that consisted of 5 μL of bestar TM PCR Master Mix buffer (Beijing ComWin Biotech Co., Ltd.), 0.45 μL of PCR primers (0.1 mM), 0.25 μL of probes (0.1 mM), and 30 ng of template DNA. All amplification and detection were conducted in 96-well PCR plates using a Bio-Rad iQ5 Real-Time PCR Detection system (Bio-Rad).

The reaction cycle was performed in two stages: 10 min at 95°C for a denaturation step, followed by 40 cycles of 15 s at 92°C and 1 min at the appropriate annealing temperature. After the PCR was completed, allelic discrimination was analyzed using the Bio-Rad iQ5 2.0 Standard Edition Optical System Software (Bio-Rad). All the samples were successfully genotyped, and 10% of them were conducted again randomly to make sure that the results were correct and identical.

Table 1. Information of Single-Nucleotide Polymorphisms Analyzed in This Study

Chr.	*Gene*	*SNP ID*	*Region*	*Polymorphism*
7p12	*EGFR*	rs2072454	Exon3	C/T
		rs2227983	Exon9	A/G (Thr/Met)
		rs17337023	Exon12	A/T
		rs1050171	Exon15	A/G
		rs2293347	Exon19	A/G
		rs884225	3′UTR	A/G
4q25	*EGF*	rs11569017	Exon15	A/T (Val/Asp)
		rs4444903	5′UTR	A/G

The SNP information is based on the database http://www.ncbi.nlm.nih.gov/SNP/

The mutation for genotypes on extrons except for rs22227983 and rs11569017 were synonymous mutations.

SNP, single-nucleotide polymorphism; *EGF*, epidermal growth factor; *EGFR*, epidermal growth factor receptor.

Statistical analysis

We used a method of the χ^2 test to detect whether the distributions of SNPs were in the Hardy–Weinberg equilibrium (HWE). Difference of allele frequencies and genotype distribution between case and control were tested using a 2×2 or 2×3 contingency tables. The Statistical Package for the Social Sciences, version 12.0 was used to analyze all the statistics, and $p<0.05$ was the criterion for statistical significance. We used the SHEsis online software (Shi and He, 2005) to acquire LD values (r^2, D′) and the haplotype estimation.

Results

Genotyping experiments were conducted for the eight SNPs. Genotype distributions of the studied SNPs were in HWE except for the SNP rs4444903 ($p<0.05$) in the *EGF*. The frequencies of case and control in each group are shown in Table 2. The frequency of the T allele of rs11569017 in *EGF* was 20.9% in HCC patients versus 15.6% in control ($p=0.001$, odds ratio [OR] = 1.43, 95% confidence interval [CI] = 1.15–1.79), and the distribution of the genotype was also significantly different ($p=0.005$). Meanwhile, the frequency of the C allele of SNP rs2072454 in *EGFR* was 61.1% in HCC patients and 56.1% in controls ($p=0.023$, OR = 1.23, 95% CI = 1.03–1.47) and the genotype distribution was different as well ($p=0.039$). We used a stepwise forward selection of binary logistic regression to determine independent factors such as age and gender for susceptibility to HCC under an additive model in all SNPs. The result showed that rs11569017 was still independently associated with the susceptibility to HCC ($p=0.021$, OR = 1.48, 95% CI = 1.06–2.07), but the SNP rs2072454 ($p=0.143$, OR = 1.21, 95% CI = 0.94–1.56) and other SNPs we chose were not associated with the susceptibility to HCC.

The degrees of LD for six SNPs in *EGFR* and two SNPs in *EGF* were examined, and no apparent LD ($r^2=0.09$ for *EGF*, $r^2\leq0.686$ for *EGFR*) was found. Then, we carried out a haplotype analysis of these SNPs, the result showed that the frequency of haplotype T-G consisted of rs11569017 and rs4444903 in the *EGF* was significantly higher in the case group than in the control group (20.9% vs. 15.4%, $p=0.002$, OR = 1.44, 95% CI = 1.15–1.82) (Table 3). However, there was no significant difference between the case and control groups in other haplotypes. These results were in accordance with the single locus analysis, suggesting that rs11569017 was a principal genetic factor in the *EGF*. No significant difference between the case group and control group in haplotypes of the *EGFR* gene in our study was found.

Discussion

The interaction between the EGFR and EGF activates the signal pathway of c-Src, PI3K, and MAPK downstream, which increases growth, migration, and adhesion as well as inhibits apoptosis. The EGFR in the tumor endothelial cells may play a role for tumor angiogenesis in HCC via paracrine mechanisms (Moon *et al*., 2006), and apparent EGF overexpression is detected in the serum of HCC patients (Abu Dayyeh *et al*., 2011), both demonstrate that the EGF and EGFR play an important role in the process of tumorigenesis. Yoneda *et al.* (2011) performed the experiment to investigate the mechanism underlying the development of cytokeratin19-positive HCC, the results indicate that the activation of the EGF-EGFR signaling pathway is associated with the development of cytokeratin 19-positive HCC, and the EGF-induced increase in growth abilities of HCC may account for the poor prognosis of the patients. Genetic variation may be associated with tumor occurrence. An association study about gastric cancer in the Chinese Han population shows that the A allele of *EGF* rs4444903 is a protective factor of gastric cancer, but the T allele of *EGFR* rs17337023 is a risk factor (Yang *et al*., 2012). In a Caucasian population, the G allele of rs4444903 is confirmed to be closely related to the incidence of esophageal cancer (Menke *et al*., 2011).

Additionally, some studies reveal the effect of the rs2293347 polymorphism in *EGFR* on the clinical efficacy of gefitinib in patients with nonsmall cell lung cancer (Ma *et al*., 2011). *EGFR* intron 1 (CA)n polymorphism effects the outcome in advanced lung cancer patients treated with EGFR-TKI (Nie *et al*., 2011). Association studies on polymorphisms of the *EGF* gene and HCC are mainly focused on rs4444903. The study carried out by Abu Dayyeh *et al.* (2011) showed that the *EGF* genotype G/G was associated with increased risk for HCC, and differences in its frequency among black and white subjects might account for differences in HCC incidence between these ethnicities (Menke *et al*., 2011). The genotype of rs4444903 in the *EGF* is associated with a higher risk of chronic HBV-infected HCC in the Chinese population (Abu Dayyeh *et al*., 2011). In 2009, a study certificated that there was no association of *EGF* rs4444903 and HCC in Chinese patients with chronic HBV infection (Qi *et al*., 2009). The researches above perceive that the association between *EGF* rs4444903 and the risk of HCC is still controversial and ambiguous. This situation may be due to facts such as ethnic diversity, control selecting, and small sample size in the Chinese Han population. Association studies on other SNPs in the *EGF* gene and liver cancer susceptibility were rarely reported. EGFR inhibitor drugs had been designed to block the signaling pathway through inhibiting the development of tumor (Kim and Lim, 2011), however, relatively few studies focused on the association between polymorphisms in *EGFR* and HCC susceptibility. Xie *et al.* (2012) elucidated the association of four polymorphisms of key molecules in the JAK/STAT signaling pathway with susceptibility to HCC, and (AG + GG) in rs11543848 of *EGFR* had a decreased risk of HCC in women.

The *EGF* located in 4q25 encodes a member of the EGF superfamily. The encoded protein is synthesized as a large precursor molecule that is proteolytically cleaved to generate the 53-amino acid EGF peptide. This protein acts as a potent mitogenic factor that plays an important role in the growth, proliferation, and differentiation of numerous cell types. A study (Liu *et al*., 2012) shows that the EGF as a mitogen can induce AKR1B10(aldo-keto reductase 1B10), which is overexpressed in liver and lung cancer, and plays a critical role in tumor development and progression by promoting lipogenesis and eliminating cytotoxic carbonyls. Rs11569017 on exon15 in *EGF* is a nonsynonymous SNP (Val/Asp), as we all know that when a nonpolar aliphatic amino acid becomes an acidic amino acid, water solubility of amino acids may increase, but decrease the stability of amino acids. Numerous studies have shown that the change of amino acid would lead to a variation of structural stability (Rabbani *et al*., 2012); some amino acid change in a special position may also lead to an enhanced signaling to reinforce protein expression (Liu *et al*.,

Table 2. Genotype Distributions and Allelic Frequencies of Single-Nucleotide Polymorphisms in *EGF* and *EGFR*

Gene	*SNPs*	*Genotype/ allele*	*Case*	*Control*	χ^2	p/p*	*OR*	*95% CI*
EGFR	rs2072454		*n = 410 (%)*	*n = 634 (%)*				
		CC	158 (38.5)	196 (30.9)	6.48	0.039/0.143	1.21	0.94–1.56
		CT	185 (45.1)	319 (50.3)				
		TT	67 (16.3)	119 (18.8)				
		C	501 (61.1)	711 (56.1)	5.16	0.023	1.23	1.02–1.48
		T	319 (38.9)	557 (43.9)				
	rs2227983		*n = 402 (%)*	*n = 643 (%)*				
		AA	113 (28.1)	170 (26.4)	1.75	0.416/0.184	1.19	0.92–1.54
		GA	205 (51.0)	316 (49.1)				
		GG	84 (20.9)	157 (24.4)				
		A	431 (53.6)	656 (51.0)	1.34	0.248	1.11	0.93–1.33
		G	373 (46.4)	630 (49.0)				
	rs17337023		*n = 393 (%)*	*n = 631 (%)*				
		AA	119 (30.3)	169 (26.8)	1.55	0.462/0.155	1.21	0.93–1.58
		TA	196 (49.9)	335 (53.1)				
		TT	78 (19.8)	127 (20.1)				
		A	434 (55.2)	673 (53.3)	0.70	0.404	1.08	0.90–1.30
		T	352 (44.8)	589 (46.7)				
	rs1050171		*n = 412 (%)*	*n = 638 (%)*				
		AA	14 (3.4)	19 (3.0)	1.35	0.256/0.115	0.76	0.56–1.07
		GA	118 (28.6)	164 (25.7)				
		GG	280 (68.0)	455 (71.3)				
		A	146 (17.7)	202 (15.8)	1.29	0.510	1.14	0.91–1.45
		G	678 (82.3)	1074 (84.2)				
	rs2293347		*n = 406 (%)*	*n = 640 (%)*				
		AA	37 (9.1)	60 (9.4)	0.33	0.850/0.478	1.10	0.84–1.45
		GA	176 (43.3)	266 (41.6)				
		GG	193 (47.6)	314 (49.0)				
		A	250 (30.8)	386 (30.2)	0.09	0.759	1.03	0.85–1.25
		G	562 (69.2)	894 (69.8)				
	rs884225		*n = 415 (%)*	*n = 643 (%)*				
		AA	112 (27.0)	147 (22.9)	2.50	0.286/0.194	1.18	0.92–1.52
		GA	206 (49.6)	330 (51.3)				
		GG	97 (23.4)	166 (25.8)				
		A	430 (51.8)	624 (48.5)	2.18	0.140	1.14	0.96–1.36
		G	400 (48.2)	662 (51.5)				
EGF	rs11569017		*n = 411 (%)*	*n = 636 (%)*				
		TT	15 (3.6)	16 (2.5)	10.53	0.005/0.021	1.48	1.06–2.07
		TA	142 (34.5)	166 (26.1)				
		AA	254 (61.8)	454 (71.4)				
		T	172 (20.9)	198 (15.6)	9.86	0.001	1.43	1.15–1.79
		A	650 (79.1)	1074 (84.4)				
	rs4444903		*n = 404 (%)*	*n = 623 (%)*				
		AA	45 (11.1)	76 (12.2)	1.80	0.407/0.706	0.95	0.735–1.23
		AG	153 (37.9)	256 (41.1)				
		GG	206 (51.0)	291 (46.7)				
		A	243 (30.1)	408 (32.7)	1.61	0.204	0.88	0.73–1.07
		G	565 (69.9)	838 (67.3)				

Cases: patients with HBV-related HCC.
Controls: individuals who had never been infected with HBV and in whom all HBV serum markers were negative.
*p**, *p*-Value conducted by binary logistic regression to determine age and gender for susceptibility to HCC under an additive model.
OR, odds ratio; CI, confidence interval; df = 2; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

2012). In addition, missense mutation may also affect the 3D structure and protein–protein interactions (Zhang *et al*., 2012). The length of the *EGF* gene is 100079 bp, which encodes a preproEGF with a total of 1027 amino acids. The Val to Asp mutation in amino acid 784 caused by the rs11569017 in human preproEGF is included in the region, which is homologous to the low-density lipoprotein receptor. Although rs11569017 is not located on the mature EGF, it may make a difference through influencing the expression of preproEGF or the splicing efficiency in downstream of amino acid 784 (971–1023) encodes a Ca^{2+}-binding domain, which is the most important functional structure and maintained in the mature EGF. The precursor found in the distal tubule cells will be membrane bound (Bell *et al*., 1986). The EGF precursor is

Table 3. Comparison of rs11569017-rs4444903 Haplotype Frequencies in *EGF* Between Cases and Controls

Haplotype	*Case (%)*	*Control (%)*	p	*OR (95% CI)*
AA	241 (30.1)	399 (32.3)	0.269	0.90 (0.74–1.09)
AG	392 (49.0)	641 (51.8)	0.176	0.88 (0.74–1.06)
TG	167 (20.9)	190 (15.4)	0.002	1.44 (1.15–1.82)
TA	0 (0.0)	6 (0.5)		

Case: patient with HBV-related HCC.
Control: individuals who had never been infected with HBV and in whom all HBV serum markers were negative.

synthesized as a membrane-bound protein with its NH_2-terminus external to the cell surface. A research demonstrated that the preproEGF contains a hydrophobic domain that may serve to anchor the precursor in the plasma membrane. Because of this feature, as well as the presence of a region of about 400 amino acids that is homologous to the low-density lipoprotein receptor has been suggested that preproEGF may function as a receptor for a yet unidentified ligand, which may have a biological function (Mroczkowski *et al.*, 1989). Those suggest that the mutation may affect the protein–protein interaction through the Ca^{2+}-binding domain in downstream indirectly or through the structure change in its precursor to influence the membrane binding directly. As described above, the nonsynonymous SNP (Val/Asp) may increase the susceptibility of HCC by means of protein–protein interactions through increasing water solubility of amino acids.

The haplotype analysis of the two SNPs in the *EGF* gene showed that the T-G haplotype consisted of rs11569017 and rs4444903 was associated with the increased risk of HBV-related HCC ($p = 0.002$, OR = 1.44, 95% CI = 1.15–1.82). The result provided evidence that T allele in rs11569017 played a leading role in the development of HBV-related HCC. Although the polymorphism of rs4444903 was not associated with HBV-related HCC in the present study, numerous studies identified that the G allele in rs4444903 was associated with the overexpression of *EGF*. In this study, the combination of rs11569017 and rs4444903 might increase the risk of HBV-related HCC, and this effect might be influenced by inducing the expression of the EGF (Tanabe *et al.*, 2008).

Only SNPs with MAF > 0.20 were selected in the present study. Some SNPs with MAF ≤ 0.20, which would have potential roles in association with susceptibility to HCC might be omitted. Therefore, a lower MAF cutoff should be selected to study more SNPs with more samples in further studies to confirm the association.

In summary, the study showed that there were significant differences in genotype distributions and allele frequencies of rs11569017 between the control group and case group, and the carriage of the rs11569017 T allele was associated with the susceptibility to HBV-related HCC, but no significant differences in the other SNPs were found.

Acknowledgments

We are thankful to all the subjects who participated in the present study. This work was supported by grants from the National Basic Research Program of China (973 Program) (No. 2012CB519005) and the Natural Sciences Foundation of China (NSFC, Grant No. 81101543).

Author Disclosure Statement

The authors declare that they have no competing interests.

References

Abu Dayyeh BK, Yang M, Fuchs BC, *et al.* (2011) A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. Gastroenterology 141:141–149.

Anzola M (2004) Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. J Viral Hepat 11:383–393.

Bell GI, Fong NM, Stempien MM, *et al.* (1986) Human epidermal growth factor precursor: cDNA sequence, expression *in vitro* and gene organization. Nucleic Acids Res 14:8427–8246.

Choura M, Frikha F, Kharrat N, *et al.* (2010) Investigating the function of three non-synonymous SNPs in EGFR gene: structural modelling and association with breast cancer. Protein J 29:50–54.

Dong J, Dai J, Shu Y, *et al.* (2010) Polymorphisms in EGFR and VEGF contribute to non-small-cell lung cancer survival in a Chinese population. Carcinogenesis 31:1080–1086.

Fisher DA, Lakshmanan J (1990) Metabolism and effects of epidermal growth factor and related growth factors in mammals. Endocr Rev 11:418–442.

Han C, Ma J, Zhao J, *et al.* (2011) EGFR mutations, gene amplification, and protein expression and KRAS mutations in primary and metastatic tumors of nonsmall cell lung cancers and their clinical implications: a meta-analysis. Cancer Invest 29: 626–634.

Han W, Lo HW (2012) Landscape of EGFR signaling network in human cancers: biology and therapeutic response in relation to receptor subcellular locations. Cancer Lett 318:124–134.

Kenny PA (2007) Tackling EGFR signaling with TACE antagonists: a rational target for metalloprotease inhibitors in cancer. Expert Opin Ther Targets 11:1287–1298.

Kim H, Lim HY (2011) Novel EGFR-TK inhibitor EKB-569 inhibits hepatocellular carcinoma cell proliferation by AKT and MAPK pathways. J Korean Med Sci 26:1563–1568.

Li Y, Xie Q, Lu F, *et al.* (2009) Association between epidermal growth factor 61A/G polymorphism and hepatocellular carcinoma susceptibility in Chinese patients. Liver Int 30:112–118.

Limaye PB, Bowen WC, Orr AV, *et al.* (2008) Mechanisms of hepatocyte growth factor-mediated and epidermal growth factor-mediated signaling in transdifferentiation of rat hepatocytes to biliary epithelium. Hepatology 47:1702–1713.

Liu Q, Chen H, Ojode T, *et al.* (2012) WHIM syndrome caused by a single amino acid substitution in the carboxy-tail of chemokine receptor CXCR4. Blood 120:181–189.

Liu Z, Yan R, Al-Salman A, *et al.* (2012) Epidermal growth factor induces tumour marker AKR1B10 expression through activator protein-1 signalling in hepatocellular carcinoma cells. Biochem J 442:273–282.

Ma F, Xu B, Lin D, *et al.* (2011) [Effect of rs2293347 polymorphism in EGFR on the clinical efficacy of gefitinib in patients with non-small cell lung cancer]. Zhongguo Fei Ai Za Zhi 14:642–645 [In Chinese].

Menke V, Pot RG, Moons LM, *et al.* (2011) Functional single-nucleotide polymorphism of epidermal growth factor is associated with the development of Barrett's esophagus and esophageal adenocarcinoma. J Hum Genet 57:26–32.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215.

Moon WS, Park HS, Yu KH, *et al.* (2006) Expression of betacellulin and epidermal growth factor receptor in hepatocellular carcinoma: implications for angiogenesis. Hum Pathol 37:1324–1332.

Mroczkowski B, Reich M, Chen K, *et al.* (1989) Recombinant human epidermal growth factor precursor is a glycosylated membrane protein with biological activity. Mol Cell Biol 9:2771–2778.

Mullhaupt B, Feren A, Fodor E, *et al.* (1994) Liver expression of epidermal growth factor RNA. Rapid increases in immediate-early phase of liver regeneration. J Biol Chem 269:19667–19670.

Nakajima H, Ishikawa Y, Furuya M, *et al.* (2012) Protein expression, gene amplification, and mutational analysis of EGFR in triple-negative breast cancer. Breast Cancer. [Epub ahead of print]; DOI: 10.1007/S12282-012-0354-1.

Nie Q, Yang XN, An SJ, *et al.* (2011) CYP1A1*2A polymorphism as a predictor of clinical outcome in advanced lung cancer patients treated with EGFR-TKI and its combined effects with EGFR intron 1 (CA)n polymorphism. Eur J Cancer 47:1962–1970.

Qi P, Wang H, Chen YM, *et al.* (2009) No association of EGF 5′ UTR variant A61G and hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. Pathology 41:555–560.

Rabbani B, Mahdieh N, Haghi Ashtiani MT, *et al.* (2012) *In silico* structural, functional and pathogenicity evaluation of a novel mutation: an overview of HSD3B2 gene mutations. Gene 503:215–221.

Shi YY, He L (2005) SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 15:97–98.

Tanabe KK, Lemoine A, Finkelstein DM, *et al.* (2008) Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. JAMA 299:53–60.

Taylor K, Gordon N, Langley G, *et al.* (2008) Estimates for worldwide laboratory animal use in 2005. Altern Lab Anim 36:327–342.

Villanueva A, Newell P, Chiang DY, *et al.* (2007) Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis 27:55–76.

Whittaker S, Marais R, Zhu AX (2010) The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene 29:4989–5005.

Xie JX, Yin JH, Zhang Q, *et al.* (2012) [Association of genetic polymorphisms of key molecules in JAK/STAT signaling pathway with susceptibility of hepatocellular carcinoma]. Zhonghua Liu Xing Bing Xue Za Zhi 33:215–219 [In Chinese].

Xin Z, Zhang W, Xu A, *et al.* (2012) Polymorphisms in the potential functional regions of the TGF-beta 1 and TGF-beta receptor genes and disease susceptibility in HBV-related hepatocellular carcinoma patients. Mol Carcinog. [Epub ahead of print]; DOI: 10.1002/mc.21876.

Yang G, Rao L, Tian L, *et al.* (2012) An association between EGF and EGFR gene polymorphisms with gastric cancer in a Chinese Han population. Hepatogastroenterology 59:2668–71.

Yoneda N, Sato Y, Kitao A, *et al.* (2011) Epidermal growth factor induces cytokeratin 19 expression accompanied by increased growth abilities in human hepatocellular carcinoma. Lab Invest 91:262–272.

Zender L, Villanueva A, Tovar V, *et al.* (2010) Cancer gene discovery in hepatocellular carcinoma. J Hepatol 52:921–929.

Zhang H, Zhai Y, Hu Z, *et al.* (2010) Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. Nat Genet 42:755–758.

Zhang Z, Miteva MA, Wang L, *et al.* (2012) Analyzing effects of naturally occurring missense mutations. Comput Math Methods Med 2012:805827.

Zhong JH, You XM, Gong WF, *et al.* (2012) Epidermal growth factor gene polymorphism and risk of hepatocellular carcinoma: a meta-analysis. PLoS One 7:e32159.

Zhu J, Meng X, Yan F, *et al.* (2010) A functional epidermal growth factor (EGF) polymorphism, EGF serum levels and renal cell carcinoma risk in a Chinese population. J Hum Genet 55:236–240.

Address correspondence to:
Ying Liu, PhD
National Laboratory of Medical Molecular Biology
Institute of Basic Medical Sciences
Chinese Academy of Medical Sciences
School of Basic Medicine
Peking Union Medical College
5 Dongdan 3 Tiao
Beijing 100005
People's Republic of China

E-mail: liuyingpumc@yahoo.com

Hui Li, PhD
Department of Epidemiology
Institute of Basic Medical Sciences
Chinese Academy of Medical Sciences
School of Basic Medicine
Peking Union Medical College
5 Dongdan 3 Tiao
Beijing 100005
People's Republic of China

E-mail: lihui99360@sohu.com