Association of Specific Genotypes in Metastatic Suppressor HTPAP with Tumor Metastasis and Clinical Prognosis in Hepatocellular Carcinoma

Ning Ren1,2,3, Jin-Cai Wu1,2,3, Qiong-Zhu Dong1,2,3, Hai-Jing Sun3, Hu-Liang Jia1,2,3, Guo-Cai Li1,2,3, Bing-Sheng Sun1,2,3, Chun Dai3, Jiong Shi3, Jin-Wang Wei3, Yuan-Yuan Sheng3, Hai-Jun Zhou1,2,3, Qing-Hai Ye1,2,3, and Lun-Xiu Qin1,2,3

Abstract

The phosphatidic acid phosphatase HTPAP has been defined as a metastatic suppressor of hepatocellular carcinoma (HCC), but little is known about its function or potential applications as a prognostic marker. In this study, we analyzed patterns of HTPAP genetic variation and gene expression in 864 patients who underwent HCC resection, assessing these patterns for correlations to tumor metastasis potential. Focusing on two tagSNPs that were selected (+357G/C and +1838A/G), we found that only the +357G/C genotype was significantly associated with HTPAP mRNA and protein expression levels and the probability of metastasis. In an independent cohort of 665 HCC patients, we determined that the +357G/C genotype was associated with shorter time to recurrence and overall survival. Together, these results indicated that the HTPAP tagSNP +357 GG+GC genotypes may influence HCC metastatic potential and clinical prognosis by down-regulating HTPAP expression. Extending these results, a global expression profiling analysis identified 41 genes including the pro-inflammatory genes IL-8 and TLR2 that were significantly overexpressed in the +357 GG+GC group, as possible coregulated markers with HTPAP. Together, our findings identify an HTPAP genotype and associated gene expression pattern that favors metastasis progression and that could be used to predict tumor metastasis and prognosis in HCC patients. Cancer Res; 71(9): 3278–86. ©2011 AACR.

Introduction

Our previous studies demonstrating that chromosome 8p deletion was associated with hepatocellular carcinoma (HCC) metastasis (1–4) identified HTPAP gene as a suppressor of in vitro invasion and in vivo lung metastasis of HCC (5). The official symbol of the HTPAP gene is phosphatidic acid phosphatase type 2 domain containing 1B (PPAPDC1B). HTPAP contains 7 exons and 8 introns; its full length is 4,459 bp with an 826-bp mRNA encoding a 175 amino acid protein. It is not known whether the presence of HTPAP genetic polymorphisms affects the prognosis of HCC patients. The mechanism of the effect of HTPAP on HCC remains unclear. Reports of potential functional roles for HTPAP in other human cancers such as breast cancer have been conflicting. Some studies suggested that HTPAP may play an oncogenic role, thus providing a new therapeutic target (6,7); however, another study reported that high HTPAP gene expression was associated with a good prognosis (8).

Recent studies report that the biological features of cancers and susceptibility to certain cancers are influenced by genetic polymorphisms and haplotypes, some of which may also be significant prognostic indicators for specific cancers such as HCC (9,10). In this study, we used a haplotype-based approach to investigate the association of specific genotypes in HTPAP with tumor metastasis and clinical prognosis in hepatocellular carcinoma.

Materials and Methods

HCC patients and tissue samples

We recruited a total of 1,529 participants (Cohort 1, n = 864; Cohort 2, n = 665), who were unrelated ethnic Han Chinese with histopathologically diagnosed HCC (The clinicopathological features are shown in Supplementary Table S1). All patients received curative liver resection without any preoperative treatment such as chemotherapy, radiotherapy, or radiofrequency ablation. We analyzed the HTPAP single nucleotide polymorphisms (SNPs) and identified haplotypes of 864 patients (Cohort 1) who underwent surgical operation...
from January 2002 to January 2003. The tagSNPs were selected and the relationship of their genotypes with HTPAP expression and tumor metastasis potential were assessed. Another independent cohort of 665 HCC patients (Cohort 2) underwent surgical operation from January 2004 to January 2005. The genotypes of tagSNP were analyzed in the tumor tissues and their adjacent noncancerous liver tissues, and also in 20 normal control liver tissues adjacent to hepatic hemangioma. The patients were followed up until January 2010, and their post-operative time to recurrence (TTR; ref. 11) and overall survival (OS) were determined. This study was approved by the ethics committees of the Liver Cancer Institute and Zhongshan Hospital, Fudan University (Shanghai, China), and we obtained written informed consent from each patient.

DNA extraction, SNP genotyping, and verification

To acquire relatively pure populations of cancer cells, laser capture microdissection (LCM) was performed on all samples with the Veritas Automated LCM System (Arcturus Bioscience). Genomic DNA from the microdissected HCC tissues was extracted with the QIAamp DNA Micro Kit (QIAGEN) according to the manufacturer’s instructions. To identify SNPs (12,13), the entire HTPAP gene (approximately 7.5 kb, including a 1.7-kb regulatory region and 1.3-kb 3’flanking region) was screened in 30 HCC specimens by direct sequencing of the PCR products of genomic DNA (ABI 3730, Applied Biosystems). Genotyping by pyrosequencing (PSQ96) was performed in the remaining HCC tissues; each 96-well assay plate contained positive and negative (no DNA) controls. The genotyping results were verified in 5% of randomly chosen samples by direct sequencing. Consistency between these 2 techniques was 99.5%, indicating the reliability of the pyrosequencing method. Repeat tests were performed in 5% of samples by different persons, and the reproducibility was 100%.

Haplotypes reconstruction and tagSNP selection

The Hardy–Weinberg equilibrium and linkage disequilibrium (LD) between polymorphisms were determined with the Haploview program (version 3.2; ref. 14). Haplotypes were analyzed with PHASE software v2.1 (15). Haplotype blocks were defined as $D’ > 0.8$, and tagSNPs were selected with Haplovie 3.2 software on a block basis.

We selected tag SNPs based on the following criteria: (i) the ability to efficiently capture all common SNPs observed in the region with a high correlation ($r^2 ≥ 0.8$) from generated pairwise LD panel (16); (ii) the predictive ability of either unmeasured SNPs or SNP haplotypes (17); and (iii) The likely power to detect the influence of common disease-causing variants in candidate genes upon the disease risk in a haplotype-based analysis (18, 19).

Analysis of HTPAP, IL-8, and TLR-2 expression

Total RNA was isolated from 377 HCC tissue specimens of Cohort 1 and used to synthesize cDNA with oligo(dT)15 primers and Superscript II (Invitrogen Life Technologies). The mRNA levels of HTPAP, interleukin-8 (IL-8), and toll-like receptor-2 (TLR-2) were determined by reverse transcriptase PCR (RT-PCR) and real-time quantitative RT-PCR (qRT-PCR) with SYBR Green PCR Master Mix in an ABI 7700 (Applied Biosystems). Primers used for qRT-PCR and RT-PCR amplification are shown in Supplementary Table S2. Each assay was performed triplicate. The mRNA levels of HTPAP were also detected in 312 HCC tissues randomly selected from Cohort 2.

Immunohistochemical staining of HTPAP

Affinity-purified antibodies were generated by immunizing a rabbit with a peptide corresponding to full-length HTPAP. Immunohistochemical staining for HTPAP was performed in archival formalin-fixed, paraffin-embedded HCC tissues. Histological sections (4 μm thick) were deparaffinized and rehydrated in graded alcohols. Then, they were immersed in methanol containing 3% H2O2 at room temperature for 10 minutes and rinsed in PBS (pH 7.4). An antigen retrieval step was performed routinely by microwave oven with 10 mmol/L sodium citrate buffer at 95°C for 10 minutes. After treatment with blocking buffer (2% normal goat serum in PBS) at 37°C for 30 minutes, the tissue sections were incubated at 4°C overnight with rabbit polyclonal antibody against human HTPAP (1:2,000) or control rabbit IgG (Sigma-Aldrich), then incubated with biotinylated anti-rabbit IgG (Santa Cruz Biotechnology) followed by Vectastain Elite ABC reagents (Vector) at 37°C for 30 minutes. Tissue sections were finally visualized with 3,3’-diaminobenzidine tetrahydrochloride/Tris-HCl buffer containing 0.01% H2O2 and counterstained lightly with Mayer’s hematoxylin. Semiquantitative analysis of immunohistochemical staining was performed by 2 experienced pathologists in 2 sections of each specimen with 10 fields of each section (magnification, 200×). Immunostaining scoring was based on the intensity of staining and the percentage of cells that stained positively: negative (−), 0% to 5%; intermediate (+), 5% to 10%; moderate (++) , 10% to 25%; strong (+++), >25%. HTPAP staining ≥5% was considered positive.

Gene expression profiling of HCC tissues

The Affymetrix Human Genome U133 Plus 2.0 Array was used for gene expression analysis. This array contains more than 47,000 transcripts representing 38,500 well-characterized human genes. This array is the most comprehensive whole human genome expression array and comprises more than 54,000 probe sets and 1,300,000 distinct oligonucleotide features. Eleven pairs of oligonucleotide probes were used to determine the transcription level of each sequence. Significance analysis of microarrays (SAM; ref. 20) was used to identify genes differentially expressed between predefined groups. Statistical significance was confirmed with the permutation distribution of the t statistic based on 2,000 random permutations. Unsupervised hierarchical clustering analysis was carried out by CLUSTER (version 2.11) and TREEVIEW (version 1.60; ref. 21) with uncensored correlation and average linkage.

Statistical analyses

Unconditional logistic regression was used to determine the association between genotypes and metastatic potential, after
adjusting for clinicopathologic characteristics. Kruskal–Wallis 1-way ANOVA tests were performed to analyze HTPAP expression. One-way ANOVA was used to assess the differences in luciferase reporter activity. Fisher’s exact test or the Wilcoxon rank sum test was used to determine correlations between HTPAP genotypes and the clinical features of HCC. For ordered variables, a test for trend (P trend) was performed. The primary outcome was time to recurrence (TTR), which was calculated as the time from treatment to HCC recurrence. A diagnosis of recurrence was based on typical appearance in computed tomography (CT) and/or MRI scan. The second outcome was OS, which was calculated as the time from cancer diagnosis to HCC-related death or study endpoint. Kaplan–Meier method and log-rank test were used to compute TTR and OS rates. Cox regression model was applied to evaluate the effect of each clinical variable and the tagSNP genotype on TTR or OS. HRs for significant tagSNP genotype were calculated with adjustments for clinical variables important for survival. Statistical analyses were performed with Statistic Analysis System software (version 8.0, SAS Institute). *P* < 0.05 was considered statistically significant, and all statistical tests were 2-sided.

**Results**

### SNPs genotyping, haplotypes reconstruction, and tagSNPs selection

After SNP detecting and genotyping of 864 HCC tissues, we identified 6 SNPs: −1053 A/G (rs3739252) on the 5′-flanking regulatory region, +64G/C in exon 1 (5′-UTR), +357G/C (rs1149) in intron 2, +1648/TAAG (rs380326, the insertion allele of TAAG was named G, and the deletion was named T) in intron 4, +1838A/G (rs11539529) in exon 5 (coding, nonsynonymous P83S), and +3528C/T (rs7007097) in intron 6. Pairwise LD measures confined the HTPAP region within a haplotype block (Haploview3.2; Fig. 1). Five SNPs (−1053 A/G, +64G/C, +357G/C, +1648 T/G, and +3528C/T) were in strong LD (r² = 0.85–1.0), with complete LD between −1053 A/G and +64G/C. Another SNP at +1838A/G showed historical recombination and was much more weakly correlated with the others (r² = 0.32–0.36).

On the basis on these 6 SNPs, a total of 11 haplotypes were identified with PHASE 2.1 software (Supplementary Table S3). The 4 most common haplotypes (frequency >4%) were AGCTAC, GCCGGT, AGCTGC, and GCAGGT, representing the alleles of −1053, +64, +357, +1648, −1838, and +3528, respectively; these haplotypes were found in 793 (92%) of the 864 HCC specimens. Moreover, most of the 793 patients had homozygous haplotypes AGCTAC (276/793, 34.8%) or GCCGGT (73/793, 9.2%), or had heterozygous haplotypes AGCTAC/GCGGGT (230/793, 29.0%), AGCTAC/AGCTGC (102/793, 12.9%), or AGCTAC/GCGGAT (72/793, 9.1%).

The +1838A/G was selected as tagSNP because it had a historical recombination and was much more weakly correlated with other SNPs. The other 5 SNPs within LD block were strongly correlated with each other, anyone of them can represent the others in the association study. We selected the SNP +357G/C as tagSNP because it was verified in ethnic Han Chinese and was the only 1 double-hit SNP of HTPAP according to the dbSNP database. These 2 tagSNPs could provide indirect information for all 6 SNPs within the LD block and sufficiently distinguish the 4 common haplotypes (22, 23).

### The association of different tagSNPs genotypes with the expression levels of HTPAP

The mRNA levels of total HTPAP and its 2 known isoforms, HTPAP A and B [National Center for Biotechnology Information (NCBI) database] of 377 randomly selected patients in Cohort 1 were determined by qRT-PCR. The HTPAP mRNA levels in HCC specimens with +357 GG+GC genotypes were significantly lower than those with CC genotype (P = 0.009). The HTPAP mRNA levels in HCC specimens with +1838 AG+GG genotypes were also lower than those with +1838 AA genotype, however, the difference did not reach the

![Figure 1. The linkage disequilibrium (LD) pattern for HTPAP gene detected in 864 patients (Cohort 1) with hepatocellular carcinoma (HCC). The haplotype block structure, as depicted by Haploview, is shown. The value within each diamond represents the pairwise correlation between the 6 single nucleotide polymorphisms (measured as D' and *r*) defined by the upper left and the upper right sides of the diamond. The diamond without a number corresponds to D’ (*r*) = 1. A, shading represents the magnitude and significance of pairwise LD, with a red-to-white gradient reflecting higher to lower D’ values. B, white to black box shading changes according to increasing *r*, with black representing strong LD.](image-url)
The association of tagSNPs genotypes with tumor metastasis potential

Based on the clinico-pathological features, the 864 patients with HCC were divided into 2 groups: metastatic (M) group including 453 cases with intrahepatic metastasis and/or vascular invasion, and nonmetastatic (NM) group including 411 cases without intrahepatic metastasis or vascular invasion. As shown in Table 2, the allele frequencies at +357G/C were significantly different in M group compared with NM group (P = 0.0009). Significant higher frequencies of GC and GG genotypes were found in the M group compared with the NM group. (For GC: 45.9% vs. 34.8%, P = 0.0001; OR = 1.77; 95% CI, 1.32–2.36. For GG: 11.7% vs. 7.1%, P = 0.003; OR = 2.11; 95% CI, 1.28–3.48.) These suggest that +357 GG+GC genotypes were closely related to an increased probability of metastasis (P = 0.002). In the multivariate regression analysis, the association of +357 GG+GC genotypes on HTPAP with HCC metastasis was independent of age, sex, HBsAg status, liver cirrhosis, serum AFP level, Edmondson grade, tumor size, and TNM stage. In contrast, at +1838A/G, no significant difference (P = 0.28) in genotype distribution was observed between the M and NM groups. Because tagSNP +1838 genotypes did not show significant relationship with HTPAP expression and tumor metastasis potential, we choose tagSNP +357G/C only to perform survival and mechanism analyses.

The association of HTPAP +357G/C genotypes with prognosis of HCC patients

The associations of HTPAP +357G/C genotype with TTR and OS were investigated in the 665 HCC patients of Cohort 2. We detected the genotype frequencies of +357G/C in the tumor tissues and their adjacent noncancerous liver tissues,
and also in 20 normal control liver tissues adjacent to hepatic hemangioma. We found GG+GC genotypes frequency in tumor tissues (50%) was higher than that in adjacent liver tissues (48%) and normal liver tissues (44%), however, the difference did not reach the statistic significance ($P = 0.258$). Compared the tumor tissues with its corresponding adjacent liver tissues, 89% of coincidence was found in genotyping of tagSNP +357G/C. Kaplan–Meier analysis showed that patients with +357 GG+GC genotypes exhibited shorter TTR and shorter postoperative OS ($P < 0.001, P < 0.001$) compared with +357 CC genotype (Fig. 3). The HR for postoperative TTR was 2.17 (95% CI = 1.56–3.03; $P < 0.001)$ and for postoperative OS was 2.29 (95% CI = 1.64–3.20; $P < 0.001)$. When the HR for +357G/C genotype was recalculated following adjustments for clinical characteristics by Cox multivariate regression analysis, the similar results were found (Table 3).

### Discussion

Cancers arise as a consequence of accumulated genetic alterations within a single cell or group of cells. The stepwise accumulation of genetic alterations drives the development and progression of malignancies, and also provides a range of nucleic acid based molecular markers for cancer prediction and diagnosis. Metastasis is a complex process that is also thought to be the result of multiple genetic changes that may be responsible for the increased abnormalities and selective survival advantages observed in metastatic lesions. Thus, to understand the molecular mechanisms of cancer metastasis, it is essential to identify the genetic alterations responsible for the metastatic potential. Analysis of gene expression profiles demonstrate that activation of genes favoring HCC metastasis is initiated in primary HCCs, and the metastatic potential is predetermined in primary tumors (24). These findings suggest that metastasis may be predicted by genetic changes in primary HCCs.

In this study, we used a haplotype-based approach to identify 6 SNPs and 11 haplotypes of HTPAP. Five SNPs ($-1053 A/G$, $+64 G/C$, $+357 G/C$, $+1648 T/G$, and $+3528 C/T$) were in strong LD ($\chi^2 = 0.85–1.0$), with complete LD between $-1053 A/G$ and $+64 G/C$, indicating that it would be sufficient for future studies.

**Table 2. The association of tagSNPs genotypes with metastasis in HCC patients of Cohort 1**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>M$^a$ group (n = 453)</th>
<th>NM group (n = 411)</th>
<th>OR$^b$ (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>+357G/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>192 (42.4)</td>
<td>239 (58.2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>208 (45.9)</td>
<td>143 (34.8)</td>
<td>1.77 (1.32–2.36)</td>
<td>0.0001</td>
</tr>
<tr>
<td>GG</td>
<td>53 (11.7)</td>
<td>29 (7.1)</td>
<td>2.11 (1.28–3.48)</td>
<td>0.003</td>
</tr>
<tr>
<td>$P_{\text{trend}}^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>199 (43.9)</td>
<td>239 (58.2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GC+GC</td>
<td>261 (57.6)</td>
<td>172 (41.8)</td>
<td>1.82 (1.38–2.40)</td>
<td>0.0002</td>
</tr>
<tr>
<td>+1838A/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>198 (43.7)</td>
<td>198 (48.2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>185 (40.8)</td>
<td>165 (40.1)</td>
<td>1.09 (0.81–1.46)</td>
<td>0.564</td>
</tr>
<tr>
<td>GG</td>
<td>70 (15.5)</td>
<td>48 (11.7)</td>
<td>1.39 (0.91–2.12)</td>
<td>0.126</td>
</tr>
<tr>
<td>$P_{\text{trend}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>198 (43.7)</td>
<td>198 (48.2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AG+GG</td>
<td>255 (56.3)</td>
<td>213 (51.8)</td>
<td>1.16 (0.88–1.52)</td>
<td>0.291</td>
</tr>
</tbody>
</table>

$^a$Number of subjects in metastatic (M) or nonmetastatic (NM) group.

$^b$Data were calculated by unconditional binary logistic regression models, adjusted for age, sex, AFP level, HBV status, liver cirrhosis, tumor size, Edmondson grade, TNM stage, etc., where appropriate. The first genotype was calculated as the reference.

$^c$Tests for trend of odds were 2-sided and based on likelihood ratio tests assuming a multiplicative model.
to examine only 1 polymorphism in this region. Another SNP at +1838A/G showed historical recombination and was much more weakly correlated with the others ($r^2 = 0.32–0.36$). The +357G/C and +1838A/G SNPs were selected as tagSNPs because of their ability to provide indirect information for all 6 SNPs within the LD block and ability to sufficiently distinguish the 4 most common haplotypes.

In this study, the tagSNP genotypes were found to be able to significantly regulate both the mRNA and protein levels of HTPAP, and the tagSNP +357GG+GC genotype could significantly downregulate HTPAP, and increase the metastatic potential of HCC. Because tagSNP +1838 genotype did not show significant relationship with HTPAP expression and tumor metastasis potential, we considered the effect of HTPAP on HCC could be investigated by examine tagSNP +357G/C only. We found no significant difference in genotype frequency of +357G/C among tumor tissues, adjacent liver tissues, and normal liver tissues, suggesting that the effect of SNP +357G/C genotype on HCC metastasis and prognosis differs according to ethnicity.

Our results found fewer HCC specimens with intrahepatic metastasis and/or vascular invasion were positive for HTPAP protein staining compared with specimens without intrahepatic spreading or vascular invasion, which further demonstrated our previous study that HTPAP is a metastatic suppressor of HCC. The +357 GG+GC genotypes were found significantly associated with lower HTPAP mRNA levels, decreased HTPAP protein expression and increased probability of metastasis. Patients with +357 GG+GC genotypes exhibited shorter TTR and shorter postoperative OS compared with +357 CC genotype. These results indicate that the HTPAP tagSNP +357 genotype in primary tumors may influence the metastatic potential of HCC and the prognosis of patients by downregulating the expression of HTPAP.

### Table 3. The association of HTPAP tagSNP +357G/C genotype with time to recurrence and overall survival of patients of Cohort 2 by Cox multivariate regression analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Time to recurrence (TTR)</th>
<th>Overall survival (OS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>$P$</td>
</tr>
<tr>
<td>Age (&gt;55 years)</td>
<td>0.93 (0.74–1.17)</td>
<td>0.543</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>1.13 (0.83–1.54)</td>
<td>0.435</td>
</tr>
<tr>
<td>HBsAg (positive)</td>
<td>0.76 (0.55–1.06)</td>
<td>0.103</td>
</tr>
<tr>
<td>Liver cirrhosis (yes)</td>
<td>0.91 (0.71–1.17)</td>
<td>0.469</td>
</tr>
<tr>
<td>Serum AFP level (≥20 ng/mL)</td>
<td>1.27 (1.00–1.62)</td>
<td>0.053</td>
</tr>
<tr>
<td>Tumor size (≥5 cm)</td>
<td>1.14 (0.91–1.43)</td>
<td>0.260</td>
</tr>
<tr>
<td>Tumor number (≥2)</td>
<td>1.48 (1.16–1.90)</td>
<td>0.002</td>
</tr>
<tr>
<td>Edmondsson grade (III–IV)</td>
<td>0.44 (0.31–0.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vascular invasion (yes)</td>
<td>2.13 (1.70–2.68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNM stage (II–III)</td>
<td>1.55 (0.94–2.54)</td>
<td>0.087</td>
</tr>
<tr>
<td>Genotypes (GG–GC)</td>
<td>1.59 (1.23–2.26)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

$^a$HRs (95% CI) and $P$ values for postoperative time to recurrence (TTR) and overall survival (OS) were adjusted according to important clinical characteristics. Survival time was defined as the period from the surgical treatment to endpoint of follow-up. The first genotype was calculated as the reference.
Global gene-expression profiling analysis revealed that 41 genes (including 2 transcripts) were significantly overexpressed in +357 GG+GC group. The expression of these genes may be coregulated by HTPAP, and they may subsequently play a role in the mechanism of HTPAP gene function, thereby influencing the metastatic potential of HCC. IL-8 (CXCL8) was the primary gene overexpressed in +357 GG+GC group, which is produced by a wide variety of cell types, including T cells, B cells, monocytes, neutrophils, fibroblasts, and endothelial cells (25, 26) and may play a role in inflammation, angiogenesis, tumorigenesis, and metastasis by activating NF-κB and MAPK in a dose- and time-dependent manner (27, 28). Overexpression of IL-8 in HCC is associated with vascular invasion and poorer prognosis (29, 30). In addition, serum IL-8 level is significantly correlated to large tumor size (>5 cm), absence of tumor capsule, vascular invasion, and advanced TNM stage (27). Strikingly, our recent study showed that, as a member of Th2 cytokine family, IL-8 was expressed at significantly higher levels in metastasis-inclined microenvironment (MIM) noncancerous samples than in metastasis-averse microenvironment (MAM) samples, producing a profound shift toward an anti-inflammatory/immune-suppressive response that promotes HCC metastasis (31, 32). TLR-2 is also overexpressed in +357 GG+GC group, which activates the NF-κB signaling pathway, thereby contributing to inflammation and cancer metastasis (33, 34). Interestingly, we found that many inflammatory/immune-related genes in the 41-gene classifier were overexpressed in +357 GG+GC group, including intercellular adhesion molecule (ICAM)-1, ICAM-2, chemokine (C-X-C motif) ligand 1 (CXCL-1, GRO-α), tumor necrosis factor (ligand) superfamily, member 13b (TNFSF13B), PACAP-responsive gene 1 (PRG1), and adhesion molecule with Ig-like domain 2 (AMIGO2). More interestingly, overexpression of many genes in the 41-gene classifier such as ICAM-1 (35,36), CXCL1 (GRO-α; 37), SOD2 (38), CTHRC1 (39), and SOCS-3 (40,41) have also been demonstrated to contribute

Figure 4. A, hierarchical clustering of 41 differentially expressed genes or transcripts with different HTPAP +357G/C genotype. Each row represents an individual gene and each column represents an individual tumor sample. Genes were ordered by uncentered correlation and average linkage according to their abundance relative to the median abundance of all genes among all tumor samples. Pseudocolors indicate differential expression (green, transcript level below the median; black, transcript level equal to the median; red, transcript level greater than the median; gray, missing data). Dendrogram was based on 16 specimens with +357 GG genotype (red, group A) and 16 specimens with +357 GC genotype (green, group B). B, qRT-PCR analysis of IL-8 and TLR-2 expression in each group. C, representative gel showing RT-PCR analysis of IL-8 and TLR-2 mRNA in each group.
in HCC metastasis or a decreased OS. These findings indicate that tagSNP +357 GG→GC genotypes of HTPAP may produce molecular signatures of metastasis, particularly involving genes associated with inflammatory/immune responses.

Our findings suggest a new way of thinking about the effects of genotypes on gene function, identify an HTPAP genotype and associated gene expression pattern that favors metastasis progression and that could be used to predict tumor metastasis and prognosis in HCC patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


12. Grant Support

This work was supported by China National Key Projects for Infectious Disease (2008ZX10002-021), the “973” State Key Basic Research Program of China (2009CB521701), Program of Shanghai Chief Scientist (08XD14008), National Natural Science Foundation of China (30600589, 30700991, 30872946), Shanghai Rising Star of Young Scientist Project (07QA14010), and The State Key Basic Research Program of China (2006CB501701).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 24, 2010; revised February 15, 2011; accepted March 7, 2011; published online April 29, 2011.


Correction: Online Publication Dates for Cancer Research May 1, 2011 Articles

The 4 articles noted below, which were published in the May 1, 2011 issue of Cancer Research, were published with incorrect online publication dates. Corrected versions of these articles have been published online.


Also, the OnlineFirst date is missing from the following article; it was published OnlineFirst on March 17, 2011.


Published OnlineFirst May 31, 2011.
©2011 American Association for Cancer Research.
doi: 10.1158/0008-5472.CAN-11-1396
Association of Specific Genotypes in Metastatic Suppressor HTPAP with Tumor Metastasis and Clinical Prognosis in Hepatocellular Carcinoma

Ning Ren, Jin-Cai Wu, Qiong-Zhu Dong, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/71/9/3278

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2011/04/22/71.9.3278.DC1

Cited articles
This article cites 35 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/71/9/3278.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.