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

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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 intratumor 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.

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 GG and 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.

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 tagSNPs 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%.

**Haplotype 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). Haplotype blocks were defined as D’ > 0.8, and tagSNPs were selected with Haploview 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 (r² ≥ 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
Results

SNPs genotyping, haplotypes reconstruction, and tagSNPs selection

After SNP detecting and genotyping of 864 HCC tissues, we identified 6 SNPs: −1053 A/G (rs739252) on the 5′-flanking regulatory region, +64G/C in exon 1 (5′-UTR), +357G/C (rs1149) in intron 2, +1648/TAAG (rs3830326, the insertion allele of TAAG was named G, and the deletion was named T) in intron 4, +1838A/G (rs11539529) in exon 5 (coding, non-synonymous 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 ($r^2 = 0.85–1.0$), 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 ($r^2 = 0.32–0.36$).

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, GCGGGT, AGCTGC, and GCGGAT, 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 GCGGGT (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 G/G+GC genotypes were significantly lower than those with CC genotype ($P = 0.009$). The HTPAP mRNA levels in HCC specimens with +1838 A+GG genotypes were also lower than those with +1838 AA genotype, however, the difference did not reach the
Table 1. The association of tagSNPs genotypes with immunohistochemical staining of HTPAP in 377 HCC tissues randomly selected from Cohort 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Positive (n = 177)</th>
<th>Negative (n = 200)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic potential</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>97</td>
<td>87</td>
<td>0.028</td>
</tr>
<tr>
<td>High</td>
<td>80</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>+357G/C genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>103</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>GG+GC</td>
<td>74</td>
<td>116</td>
<td>0.002</td>
</tr>
<tr>
<td>+1838A/G genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>89</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>AG+GG</td>
<td>88</td>
<td>115</td>
<td>0.130</td>
</tr>
</tbody>
</table>

Abbreviation: IHC, immunohistochemical staining.

The association of HTPAP +357G/C 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, found between tagSNP +1838 genotypes and the expression of HTPAP (P = 0.130; Table 1).

Specific Genotypes in HTPAP and Prognosis of HCC Patients

statistic significance (P = 0.059; Fig. 2). These results were further verified in 312 HCC tissues randomly selected from the Cohort 2. The HTPAP mRNA levels in HCCs with +357 GG+GC genotypes were found to be significantly decreased compared with those with CC genotype (P = 0.014) and HTPAP levels in HCCs with +1838 AA+GG genotypes were also lower than those in +1838 AA HCCs, but the difference did not reach the statistic significance (P = 0.072). These suggest that the findings from Cohort 1 were repeatable and convincing. (Supplementary Fig. S1).

Positive immunohistochemical staining for HTPAP were observed in 177 of 377 randomly selected patients in Cohort 1 (46.9%). Moreover, fewer HCC specimens with intrahepatic metastasis and/or vascular invasion (including portal and hepatic vein, or bile duct) were positive for HTPAP protein staining compared with specimens without intrahepatic spreading or vascular invasion (41.5% vs. 52.7%, P = 0.028). The association of tagSNPs genotypes with HTPAP protein expression was also analyzed; the +357 GG+GC genotypes were significantly associated with decreased HTPAP expression (P = 0.002). However, no significant relationship was
Table 2. The association of tagSNPs genotypes with metastasis in HCC patients of Cohort 1

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>M* group (n = 453)</th>
<th>NM group (n = 411)</th>
<th>OR* (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>+357G/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>192</td>
<td>42.4</td>
<td>239</td>
<td>58.2</td>
</tr>
<tr>
<td>GC</td>
<td>208</td>
<td>45.9</td>
<td>143</td>
<td>34.8</td>
</tr>
<tr>
<td>GG</td>
<td>53</td>
<td>11.7</td>
<td>29</td>
<td>7.1</td>
</tr>
<tr>
<td>P_trend</td>
<td></td>
<td></td>
<td>0.0009</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>199</td>
<td>43.9</td>
<td>239</td>
<td>58.2</td>
</tr>
<tr>
<td>GC+GC</td>
<td>261</td>
<td>57.6</td>
<td>172</td>
<td>41.8</td>
</tr>
<tr>
<td>+1838A/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>198</td>
<td>43.7</td>
<td>198</td>
<td>48.2</td>
</tr>
<tr>
<td>AG</td>
<td>185</td>
<td>40.8</td>
<td>165</td>
<td>40.1</td>
</tr>
<tr>
<td>GG</td>
<td>70</td>
<td>15.5</td>
<td>48</td>
<td>11.7</td>
</tr>
<tr>
<td>P_trend</td>
<td></td>
<td></td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>198</td>
<td>43.7</td>
<td>198</td>
<td>48.2</td>
</tr>
<tr>
<td>AG+GG</td>
<td>255</td>
<td>56.3</td>
<td>213</td>
<td>51.8</td>
</tr>
</tbody>
</table>

*Number of subjects in metastatic (M) or nonmetastatic (NM) group.

bData 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.

cTests for trend of odds were 2-sided and based on likelihood ratio tests assuming a multiplicative model.

The potential mechanism of the HTPAP +357G/C genotypes in HCC metastasis

To evaluate potential mechanisms of the HTPAP +357G/C genotype in HCC metastasis, the gene-expression profiles of 16 HCC specimens selected randomly from the +357 GG+GC group were analyzed compared with 16 specimens selected from the +357 CC group. Gene expression was analyzed with Affymetrix Human Genome U133 Plus 2.0 Arrays, which include over 47,000 transcripts representing 38,500 genes. By SAM analysis, 41 genes (including 2 transcripts) were found to be significantly overexpressed in +357 GG+GC group (P < 0.05, fold change ≥ 2; Supplementary Table S4), and +357 GG+GC specimens could be accurately distinguished from CC specimens by hierarchical clustering analysis with uncensored correlation and average linkage (88% accuracy, with 4 specimens misclassified; Fig. 4A). Within the 41-gene classifier, expression levels of the 2 most important genes, IL8 (fold change > 8) and TLR-2 (fold change > 3) was confirmed by RT-PCR and qRT-PCR (Fig. 4B and C). Further, IL8 and TLR-2 were more highly expressed in specimens with GG+GC genotypes.

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, +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, indicating that it would be sufficient for future studies.
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 tagSNPs 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.

![Figure 3. Kaplan–Meier analysis of time to recurrence (TTR) and overall survival (OS) of patients of Cohort 2 with different genotypes.](image)

**Figure 3.** Kaplan–Meier analysis of time to recurrence (TTR) and overall survival (OS) of patients of Cohort 2 with different genotypes. A, earlier TTR was associated with +357GG genotype. A, earlier TTR was associated with +357GG genotype. A, earlier TTR was associated with +357GG genotype. A, earlier TTR was associated with +357GG genotype.

**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 (≥55years)</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>Edmondson 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>

*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-kB 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-kB 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), CT1HC1 (39), and SOCS-3 (40,41) have also been demonstrated to contribute
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

3. Pang JJZ, Qin LX, Ren N, Hei ZY, Ye QH, Jia WD, et al. Loss of heterozygosity at D8S298 is a predictor for long-term survival of patients with tumor-node-metastasis stage I of hepatocellular carcino-

7. Bernard-Pierrot I, Gruet N, Stransky N, Vincent-Salomon A, Reyal F, Department of Statistics at University of Washington [Internet]. Seattle:

13. National Center for Biotechnology Information [Internet]. Bethesda: National Center for Biotechnology Information [Internet]. Bethesda:

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.


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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.


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