Changes in glucose-6-phosphate dehydrogenase expression results in altered behavior of HBV-associated liver cancer cells

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Hepatocellular carcinoma (HCC) is regarded as a major global health care issue. Newly diagnosed HCC patients are expected reach about 564,000 each year (4, 6). Because of the prevalence of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, hepatocellular carcinoma is particularly common in Asia. In China, since the 1990s, HCC has been the second most fatal cancer (42), and there it is mainly caused by HBV infection. Although significant progress has been made in the treatment of HCC, poor treatment success and the high incidence of tumor continue to be major challenges (15). Because of a lack of effective therapy, HCC has one of the worst mortality rates among the common malignancies (29, 30). Accordingly, it is urgent to find additional and effective molecular targets for the treatment of HCC.

Proteomic analysis is a useful technology for the evaluation of protein expression. Moreover, it has been extensively applied to neoplastic diseases (41). Two-dimensional electrophoresis (2-DE) is a primary proteomic-based technology that has been applied to compare protein expression levels in HCC. Kuramitsu et al. (26) used 2-DE plus MALDI-TOF/MS to demonstrate that tubulin alpha-6 chain is a potential biomarker for well-differentiated HCV-associated HCC. On the basis of 2-DE of HCC and adjacent non-HCC tissue specimens, SULT1A1 was reported to be helpful in the discovery of early HCC and assisted in the prediction of the clinical outcome of patients with HCC (52). However, 2-DE has drawbacks: intergel variation, low sensitivity, excessive time, and high labor costs (19, 41).

Recently, several stable isotope technologies have been developed, including iTRAQ, for the quantitation of peptides in proteomic studies. The iTRAQ reagents can label eight samples in one experiment, enabling simultaneous identification and quantitation. As such, iTRAQ is an especially efficient and productive technology. It has been used to identify and accurately quantitate alterations of paired tissues from HBV-related HCC patients. In that study, all HCC samples were moderately differentiated with Edmondson grade II or III. The aim of the present study was to use iTRAQ and LC-MS/MS to identify novel proteins associated with HCC that may be potential targets for future treatment.

MATERIALS AND METHODS

Reagents. Eightplex iTRAQ reagent kits were obtained from Applied Biosystems (Foster City, CA). Amersham Biosciences (Uppsala, Sweden) supplied the enhanced chemiluminescence (ECL) system consisting of IgG antibodies coupled with horseradish peroxidase (HRP). Glucose-6-phosphate dehydrogenase (G6PD)-specific siRNA (HSS103891, HSS103892, HSS103893), the negative control siRNA (12935–400), and Lipofectamine 2000 were obtained from Invitrogen (Carlsbad, CA). Monoclonal or polyclonal antibodies against ADH1B, G6PD, HSPB1, APOE, S100A11, villin-1, HBsAg, and actin were purchased from Abcam (Cambridge, MA).

Tissues and cells. Approval was obtained from the local Ethics Committee before the start of study. Written, informed consent was obtained from all patients, who were verified as negative for hepatitis virus C (HCV) and human immunodeficiency virus (HIV). HBV-related HCC and adjacent non-HCC tissue specimens used for iTRAQ combined LC-MS/MS were obtained from nine HBV-related HCC patients who had undergone hepatectomy (Table 1). With regard to HCC patient characteristics in Table 1, and the association with HBV,
all patients were documented to have had serum HBsAg and confirmed to have had detectable serum HBV DNA, prior to the diagnosis of HCC. The human hepatoma cell lines HepG2, HepG2.2.15 (ATCC), and BEL7402 (Cancer Research Department of China Medical Science Institute) were cultured in high-glucose DMEM containing 10% fetal bovine serum (GIBCO, San Diego, CA) at 37°C under 5% CO2. Human immortalized normal liver L02 cells were transduced with pcDNA3.1-HBV plasmids [kindly provided by Professor Guo Hui (Chongqing Medical University, Chongqing, China)] followed by G418 selection. L02 cells transfected with pcDNA3.1-HBV plasmids were quantified by tandem mass spectrometry with ratios of the peak areas under 113–116 Da. These values corresponded to the masses of iTRAQ tags applied to label the samples. ProteinPilot software was used utilizing the embedded Paragon Algorithm. Confidence limits of 95% were used for both identification and quantitative analysis. At least two unique peptides were required for protein identification. P values were required to be <0.05 for relative quantification by iTRAQ. Proteins not meeting these criteria were excluded from the analysis.

### Table 1. Clinical and pathological data of HCC patients

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Age, yr</th>
<th>Gender</th>
<th>Edmonds on Grade</th>
<th>AFP, ng/ml</th>
<th>Tumor Size, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>M</td>
<td>II</td>
<td>6.77</td>
<td>5.0 ± 3.5</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>M</td>
<td>III</td>
<td>789</td>
<td>4.4 ± 4.6</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>F</td>
<td>II</td>
<td>378</td>
<td>2.5 ± 3.0</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>F</td>
<td>III</td>
<td>4.34</td>
<td>4.1 ± 3.3</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>F</td>
<td>III</td>
<td>1210</td>
<td>3.9 ± 2.8</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>F</td>
<td>II</td>
<td>6.09</td>
<td>2.5 ± 3.4</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>M</td>
<td>III</td>
<td>1128</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>8</td>
<td>81</td>
<td>M</td>
<td>II</td>
<td>3.3</td>
<td>2.9 ± 2.2</td>
</tr>
<tr>
<td>9</td>
<td>43</td>
<td>M</td>
<td>II</td>
<td>1.93</td>
<td>8.5 ± 6.6</td>
</tr>
</tbody>
</table>

M, male; F, female; AFP, α-fetoprotein. HCC cells compared with the control group, *P < 0.01.

### Table 2. Primers were applied to detection of transcription level of IFN type I, as well as the IFN-induced genes

<table>
<thead>
<tr>
<th>Primers</th>
<th>Description</th>
</tr>
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<tbody>
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<td>IFNAl</td>
<td>(Hs00855471_g1)</td>
</tr>
<tr>
<td>INFBl</td>
<td>(Hs01079758_s1)</td>
</tr>
<tr>
<td>ISG15</td>
<td>(Hs01921425_s1)</td>
</tr>
<tr>
<td>Mx1</td>
<td>(Hs00896508_m1)</td>
</tr>
<tr>
<td>OAS1</td>
<td>(Hs00973637_m1)</td>
</tr>
<tr>
<td>OAS2</td>
<td>(Hs00942643_m1)</td>
</tr>
<tr>
<td>OAS3</td>
<td>(Hs00196324_m1)</td>
</tr>
<tr>
<td>OASL</td>
<td>(Hs00984390_m1)</td>
</tr>
<tr>
<td>RNASel</td>
<td>(Hs00221692_m1)</td>
</tr>
<tr>
<td>EIF2a</td>
<td>(Hs00230684_m1)</td>
</tr>
</tbody>
</table>

Mx1, Myxovirus genus X-1; OAS, oligoadenylate synthetase; RNASel, RNA-specific endonuclease; EIF2a, eukaryotic initiation factor 2a.
Fig. 1. A: flow chart of iTRAQ proteomics approach. B: a representative MS/MS spectrum showing the peptides from glucose-6-phosphate dehydrogenase (G6PD; peptide sequence: ASLEAAIAIAAEQR). The ratio of 114:113 and 116:115 indicated the relative abundance of the G6PD protein in liver tumor tissues compared with nontumor tissues. IEF, isoelectric focusing.
Table 3. Partial list of proteins found to be expressed at different levels between noncancer and liver cancer tissues by iTRAQ analysis

<table>
<thead>
<tr>
<th>N</th>
<th>Accession No.</th>
<th>Gene Symbol</th>
<th>Protein Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peptides (95%)</td>
</tr>
<tr>
<td>1</td>
<td>IPI000942979.1</td>
<td>TKT</td>
<td>Isoform 2 of pyruvopimidine tract-binding protein 1 18</td>
</tr>
<tr>
<td>2</td>
<td>IPI00334175.3</td>
<td>PTBP1</td>
<td>Transketolase 12</td>
</tr>
<tr>
<td>3</td>
<td>IPI000479946.3</td>
<td>STIP1</td>
<td>STIP1 protein 5</td>
</tr>
<tr>
<td>4</td>
<td>IPI000549248.4</td>
<td>NPM1</td>
<td>Isoform 1 of nucleophosmin 11</td>
</tr>
<tr>
<td>5</td>
<td>IPI00032140.4</td>
<td>SERPINH1</td>
<td>Serpin H1 8</td>
</tr>
<tr>
<td>6</td>
<td>IPI000414676.6</td>
<td>HSP90A1</td>
<td>Heat shock protein HSP 90-beta 65</td>
</tr>
<tr>
<td>7</td>
<td>IPI000219301.7</td>
<td>MARCKS</td>
<td>Myristoylated alanine-rich C-kinase substrate 5</td>
</tr>
<tr>
<td>8</td>
<td>IPI00030781.1</td>
<td>STAT1</td>
<td>Isoform alpha of signal transducer and activator of transcription 1-alpha/beta 10</td>
</tr>
</tbody>
</table>

**Top 30 proteins upregulated in liver cancer tissues**

- **PTEN**
- **AKT1**
- **PI3K**

**Top 30 proteins downregulated in liver cancer tissues**

- **PIK3C3**
- **PAK1**
- **MAP2K1**
Supernatant HBV DNA analysis. HepG2.2.15 cells, which contain a complete HBV genome (37), were transfected with G6PD siRNA as described above. At 48 h posttransfection, culture media were collected for measurement of HBV DNA as described previously (43). In summary, cell debris was removed by centrifugation and core particles precipitated with polyethylene glycol 8000. After treatment with DNase, proteinase K in SDS and EDTA were added and the DNA extracted by phenol/chloroform. The HBV DNA was precipitated with ethanol, resuspended in Tris-EDTA buffer, and digested with RNase. Supernatant HBV DNA analysis.

**RESULTS**

**iTRAQ study of differential proteins.** Samples were labeled with iTRAQ tags in duplicate, to increase the confidence of the data, as well as improve the protein identification coverage. The labeling was follows: pooled nontumor tissues, 113; pooled nontumor tissues, 115; pooled tumor tissues, 114; pooled tumor tissues, 116. Figure 1A shows a schematic of the iTRAQ methodology. Figure 1B shows the G6PD MS/MS spectrum. Nine nontumor specimens were pooled followed by

this scheme, the percentage of positive cells (0–100%) was multiplied by the staining intensity (scale: 0–3) to produce a 0–300 score (21). To maintain consistency, the same certified pathologist examined all sections and was blinded to the results of the analysis and scoring of IHC data.

**G6PD RNA interference, wound healing, and invasion assays.** HepG2 and BEL7402 cell lines were resuspended in serum-free media. Lipofectamine 2000 was used to transfect the cells with 50 nM of control or G6PD siRNA. G6PD siRNA (5'-AACACCUUGACCUUCUCUAUCGGA-3', 5'-UGAAGAAACAUGCCGCUCCU-UUGGU-3', 5'-ACGAGCUAGUAAGAGAGAGUGGGU3') were used for downregulation. At 4 h posttransfection, the medium was changed and cells were grown for an additional 44 h. Invasion and wound healing experiments were performed at 48 h posttreatment as described previously (50). In brief, cell monolayers were incised with a P200 pipette tip. Cells were washed and incubated in the serum-free growth medium at 37°C. Images of the incision site were captured at 0 and 24 h by phase-contrast microscopy. Adobe Photoshop 7.0 was utilized to quantitatively measure the relative widths of the scratches. Cell migration was measured as the extent of closure of the incision site. The invasion assay was performed as described previously. Briefly, 1 × 10^5 treated cells were placed in the upper chamber separated from a lower chamber by an 8-μm pore size membrane (Cell Biolabs). Growth medium was added to the lower chamber and the plates were incubated for 24 h. Noninvasive cells were removed. The invasive cells were dissociated from the bottom, lysed, and quantified by use of CyQuant GR fluorescent dye (560 nm).

In all cases, Western blots were used to measure downregulation of gene expression. We performed all experiments in triplicate.
labeling with iTRAQ 113, 115 tags and an additional nine tumor samples were pooled followed by labeling with iTRAQ 114, 116 tags. Therefore, the G6PD protein relative abundance could be calculated from the 114:113 and 116:115 ratios (Fig. 1B) in the pooled tumor tissues with respect to the control group.

When searching the ProteinPilot-based database, we adopted a confidence limit of 95% and a false discovery rate of 5%. An additional cutoff of 1.3-fold was included for all iTRAQ ratios, despite the statistical analysis of the ProteinPilot 2.0 program. This was done to increase stringency in identifying up- and downregulated proteins and to account for inherent technical variation in methods. The threshold was obtained from calculations of the replicate iTRAQ experiments in which a rate of technical variation <30% was assumed. Other previous analyses using the iTRAQ approach have used this value, and it is well accepted (8, 9, 18, 35). Therefore, the upper and lower limits of the range were 1.3 (1 x 1.3) and 0.77 (1/1.3), respectively (54). Proteins with iTRAQ ratios below 0.77 were considered to be underexpressed, whereas those above 1.3 were deemed to be overexpressed (54). On the basis of the present system, 1,607 proteins were identified with confidence of 95% and 222 were determined as being differentially expressed comparing pooled nontumor tissues and pooled tumor tissues. Table 3 shows the leading 30 up- or downregulated ones, in pooled tumor with respect to nontumor tissue samples.

**Classification of proteins based on molecular and cellular function.** The PANTHER (Protein Analysis through Evolutionary Relationships) Classification System (www.pantherdb.org) was used to group the 222 differentially expressed proteins associated with HCC according as their reported biological process, protein class, and molecular functions. This allows better appreciation of the functional and molecular characteristics of these proteins. These proteins were found to represent a total of 16 biological processes, 25 protein classes, and 11 molecular functions. The top three biological processes categories were metabolic (34.9%), cellular (9.5%), and immune system processes (7.6%). The top three protein class categories were oxidoreductase (21.3%), transferase (15.3%), and nucleic acid binding (5.4%). The top three molecular function categories were catalytic (55.9%), binding (17.6%), and structural molecule activity (9.8%).

**Validation of differentially expressed proteins.** Western blotting and RT-PCR were utilized to measure the levels of the proteins, and mRNA, respectively as identified by iTRAQ. Figure 2A shows that HSPB1, villin-1, AKR1B10, APOE, UGDH, CSTB, G6PD, HIST2H2BF, HNRNPA1, GSN, NPM1, S100A11, S100A6, TKT, and VIL1 mRNA levels were upregulated in the liver tumor specimens. Conversely, ADH1B, ADH4, ADH1C, CYP1A2, and UGT2B4 mRNA levels were downregulated, compared with liver nontumor tissues. This result was similar to the results achieved by proteomic analysis. Immunoblotting shown in Fig. 2B. Tumor tissues showed an apparent upregulation of G6PD, APOE, S100A11, villin-1, and HSPB1, and an obvious downregulation of ADH1B, compared with liver nontumor tissue samples.

As shown in Fig. 3, a significant number of HCC tissues expressed G6PD, HSPB1, S100A11, villin-1, and HBsAg at levels higher than those detected in the nontumor tissues. Specifically, 35/40 (88%) of HCC samples expressed G6PD, as opposed to 26/40 (65%) of nontumor samples. Moreover, the G6PD staining intensity in IHC was stronger in the HCC samples. Similar trends were seen with HSPB1, S100A11, villin-1, and HBsAg in HCC tissues compared with nontumor tissues, whereas the reverse trend was seen with ADH1B. The results reflect those found by proteomic analysis.

**Effect of HBV on levels of G6PD protein.** The amount of G6PD activity and mRNA abundance differs between tissues and G6PD expression has been found to be regulated in liver tissue (32). It has been reported that glucose-6-phosphate dehydrogenase (G6PD) activities were decreased in early pas-
sages after SV40 infection (5). G6PD differential expression in HBV-expressing cells and tissues was measured by Western blot and normalized against control groups, to evaluate the correlation of HBV infection and G6PD expression. To our surprise, G6PD was upregulated in the HBV-expressing cell line, HepG2.2.15, compared with its parent non-HBV-expressing cell line, HepG2 (Fig. 4A). As shown in Fig. 4B, G6PD protein was markedly overexpressed in LO2/pcDNA3.1-HBV (LO2-HBV) cells compared with LO2/pcDNA3.1 [LO2-(−)] cells. Figure 4C reveals that, compared with normal liver tissues, G6PD protein was also overexpressed in HBV-infected liver tissues.

Correlation between G6PD and HBV replication. Literature searches of analyses of the effects of upregulation and G6PD function in viral infection, combined with bioinformatic analysis, permitted us to hypothesize that G6PD is involved in HBV replication. To test this hypothesis, three G6PD and a control siRNA were employed to transfect HepG2.2.15 hepatoma cell line. Each of the G6PD-specific sequences effectively silenced expression of G6PD. As expected, the control siRNA did not silence expression of G6PD (Fig. 5A). We then subjected the control and the three types of G6PD knockdown HepG2.2.15 cells to PCR to quantitate the HBV DNA. As shown in Fig. 5B, siRNA-mediated silencing of G6PD led to a fivefold reduction in HBV production, compared with untreated controls. Additionally, secreted HBsAg and HBeAg concentrations in the supernatants were also significantly decreased when G6PD was silenced.

G6PD-induced alteration of IFN signaling pathways. IFN type I and IFN-induced gene transcription after G6PD silencing was measured. G6PD-siRNA transfection increased IFN type I as well as IFN-stimulated genes (EIF2a, RNASEL, OAS3, OAS1, and ISG15) mRNA levels as shown in Fig. 5C. However, in response to knockdown of G6PD expression, IFN type I-induced antiviral signals, OASL, OAS2, and MX1 mRNA levels changed little.

G6PD is involved in HCC invasion and cell migration. The bioinformatics and experimental data suggested that G6PD is involved in HCC invasion and migration. Three G6PD and a control siRNA were used to transfect BEL7402 and HepG2 cells. G6PD expression was downregulated in G6PD siRNA-treated cells, but not in controls (Fig. 6A).

Downregulation of G6PD by siRNAs in BEL7402 and HepG2 cells decreased invasion by 35–50% and 31–39%, respectively, with respect to control (P < 0.01) (Fig. 6B). Likewise, G6PD downregulation in BEL7402 and HepG2 cells led to 46–52% and 38–45% decreases, respectively, in the time to closing of scratch wounds (Fig. 6C). The incomplete suppression might have been due to partial G6PD downregulation. Other molecules may be required in cooperation with G6PD. The proliferation of G6PD-silenced BEL7402 cells and HepG2 cells was compared with control cells by use of the MTS assay. G6PD-silenced BEL7402 cells and HepG2 cells exhibited only 8–12% and 10–12% fewer cells, respectively, compared with control groups on day 4, suggesting that G6PD takes part marginally if at all, in HCC propagation (data not shown). As shown in Fig. 6D, compared with the control cell group, expression of STAT3, y705-STAT3, MMP-9 and MMP-2 were decreased in G6PD-knockdown HCC cells.

Discussion

Several studies have used proteomic analysis of HCC cell lines (11, 16, 27, 33, 39) and animal models (2, 10, 51). However, further validation of results in patient tissue samples is needed (12) since there may be significant differences in carcinogenesis between human beings, rodents, and in vitro models.

Expression of G6PD was found to be increased in HBV-associated HCC patients, in HBV-infected patients, and in the HBV DNA-stably transfected cell line HepG2.2.15. Thus it is likely that G6PD expression is responsive to HBV infection and plays a role in its pathogenesis. As the rate-limiting enzyme of the ubiquitous pentose phosphate pathway, G6PD is a housekeeping enzyme expressed in all cells (34). G6PD overexpression has been shown to lead to increased GSH levels and to be protective against oxidant-mediated cell killing, whereas G6PD deficiency is known to cause cells to be more susceptible to oxidant attack (36). Oxidative stress is known to affect viral proliferation and virulence (14). A previous study showed that G6PD-deficient and G6PD-knocked-down cells had higher rates of viral gene expression and viral particle production than their counterparts for dengue virus serotype 2, enterovirus 71, and human coronavirus 229E (7, 20, 48). Accordingly, overexpression of G6PD or addition of an antioxidant attenuated the increased susceptibility to virus infec-

Fig. 4. Evaluation of the G6PD differential expression in HepG2 and HepG2.2.15 cells, LO2-(−) and LO2-HBV cells, normal liver, and hepatitis B virus (HBV)-infected liver tissues.

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tion (20, 48). Taken together, these previous findings indicated that oxidative stress in host cells is an important factor in virus infectivity and suggested to us that G6PD may also contribute to the process of HBV replication. Thus we examined the effects of G6PD on the HepG2.2.15 cells, which expressed HBV DNA. It was only when G6PD expression was suppressed in HepG2.2.15 cell lines that an approximately fivefold reduction in HBV was seen. In addition, G6PD suppression led to reduced concentrations of HBsAg and HBeAg in supernatant. Unlike the findings from previous studies, the present results indicate that in G6PD-knocked-down HepG2.2.15 cells, increased oxidant production caused by G6PD deficiency was not solely responsible for attenuated HBV replication. Other mechanisms are likely to be involved. In HepG2.2.15 cells, we focused on the effects on the interferon antiviral pathway with G6PD silencing. G6PD silencing resulted in an increase in type I interferon and some downstream signaling genes mediated by IFN. It has been reported that variant G6PD levels can regulate STAT3 expression. Furthermore, STAT3 has been found to negatively regulate type I IFN-induced antiviral response (22, 47). Thus our data and previous findings suggest that silencing of G6PD may increase IFN mRNA through the STAT3 pathway.

On the other hand, the pentose phosphate pathway controlled by G6PD is known for its role as a provider of ribose phosphate to the cell for nucleotide biosynthesis (44, 53). It is possible that HBV replication and the overall number of cells following G6PDH knockdown were due to a decrease in the pool of nucleotides.

G6PD is the key enzyme of the pentose phosphate pathway, which, in rapidly growing cells, generates NADPH and ribose-5-phosphate, requisites for the synthesis of RNA and DNA (3, 24). Therefore, G6PD is required for tumor cell survival and growth (17). In fact, G6PD is highly expressed in many cancers, including endometrial, colon, breast, lung, prostatic, cervical, and other cancers (1, 23). Interestingly, G6PD ectopic expression in anchorage-independent NIH3T3 cells was shown to promote cell growth (24, 25). Furthermore, in nude mice, these cells were found to be angiogenic and tumorigenic, suggesting that G6PD may act as an oncogene (17). The experimental results confirmed that the expression of G6PD was higher in HBV-associated liver cancer tissues compared with control tissues. Additionally, silencing of G6PD inhibited BEL7402 and HepG2 cell line invasion and migration, further indicating that G6PD may be a potential target for the treatment of liver cancer. In the present study, we focused on the mechanism of suppression of tumor cell motility due to silencing of G6PD. We found that the expression of STAT3, pY705-STAT3, MMP-9, and MMP-2 were decreased in G6PD-knockdown HCC cells compared with the controls. It has been reported that variant G6PD levels can regulate STAT3 expression (22). Furthermore, inhibition of activated STAT3 was found to parallel a decrease in transcriptional expression of the STAT3 target genes MMP-2 and MMP-9 and led to reduced migration and invasion activity of glioblastoma cells (22, 38). Taken together, the experimental data and previous findings indicate that silencing of G6PD may, in conjunction with the STAT3 pathway, suppress HCC cell migration and invasion.

Another interesting result from our study was that the levels of two Ca2+-binding proteins family members, including S100A11 and S100A6, were altered in HBV-associated HCC patients. S100A11 results were confirmed by quantitative RT-PCR, Western blotting, and IHC on tissue microarrays. Previous studies have shown that S100A11 was highly expressed in human gastric carcinoma and renal cell carcinoma, and S100A10 silencing resulted in partial inhibition of invasion of colorectal cancer cells (Colon 222) (13, 55). S100A11 expression has been found to be elevated in gastric cancer accompanied by lymph node metastases as opposed to those not accompanied by lymph node metastases. Upregulated protein levels were also recently discovered in HCC cell lines with high metastatic potential (11, 31). S100A11 expression was
clearly upregulated in metastatic HCC tissues compared with nonmetastatic primary HCC tissues (40). S100A11 is a likely indicator for diagnosis and target for treatment of HCC metastasis.

In this study, another protein found to be increased in HBV-related HCC samples was villin-1, an exceptionally versatile epithelial cell-specific actin-binding protein. Upregulation of villin-1 in high serum α-fetoprotein-associated HCC tumor tissues has been correlated with high serum PIVKAI11, poor differentiation, vascular invasion, an advanced stage of cancer, and the absence of recurrence. Patients with early HCC recurrence displayed upregulated villin-1 more frequently than patients with late recurrence (49). Considering the known functions of villin-1, it likely takes part in the development of HCC and could be as a marker of postoperative recurrence and poor prognosis of HCC.

Finally, we have produced a proteowide comparative profile between HBV-associated HCC and non-HCC specimens. The resulting data on HBV-associated HCC proteins provide a good resource for basic research and future applications. In addition, the evidence presented in this study describes a new role for G6PD, one that broadens the spectrum of known viruses capable of interacting with and being affected by G6PD. The data suggest that G6PD has a role in HCC cell migration and invasion. On the basis of these data, G6PD may be a novel target in future treatment of cancer of the liver.

REFERENCES
GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND HBV-ASSOCIATED LIVER CANCER


