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Original Article

Comparison of Protein Expression Patterns Between Hepatocellular Carcinoma Cell Lines and a Hepatoblastoma Cell Line

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Abstract

Hepatocellular carcinoma (HCC) and hepatoblastoma (HB) are malignancies of the liver with different etiologies, but the HB cell line HepG2 has been frequently used in various studies of HCC. In this study, we compare the protein expression patterns between HepG2 cells and three HCC cell lines, HKCI-2, HKCI-3, and HKCI-4, respectively. The cell lysates of individual cell lines were separated by two-dimensional polyacrylamide gel electrophoresis. The protein spots in the gel images were quantified and compared by image analysis software. The differentially expressing proteins were then identified by tryptic peptide mass fingerprinting. Compared with the HepG2 cells, the normalized quantities of 49 and 58 protein spots were found to be at least twofold higher and twofold lower, respectively, in all three HCC cell lines. The differentially expressed proteins can be grouped into structural proteins (annexins, transgelin, laminin receptor), stress-induced proteins (HSP27, 60, and 70), enzymes (aldehyde dehydrogenase, pyruvate kinase, α-enolase, etc.), and transcription factors (far upstream element binding protein 2, GTP-binding nuclear protein RAN).

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Some of these proteins play important roles in regulating homeostasis, drug resistance, apoptosis, cell differentiation, cell growth, and metastasis. In conclusion, our proteomic data indicate that there are considerable differences in the protein expression patterns between HepG2 cells and the HCC cells, suggesting differences in cellular properties. Hence, HepG2 may not be a good cell line model for studying HCC.

Key Words: Hepatocellular carcinoma; hepatoblastoma; proteome; two-dimensional polyacrylamide gel electrophoresis; cell line models.

Introduction

Primary liver malignancies can be grouped into different types according to the lineage of transformed cells, histology, and various pathological parameters. Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and is the third leading cause of cancer death *(1)*. About three-quarters of the cases of HCC are found in southeast Asia (China, Hong Kong, Taiwan, Korea, and Japan). In addition, HCC is also very common in sub-Saharan Africa (Mozambique and South Africa). HCCs originate from hepatocytes. It is well known that hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, liver cirrhosis, and exposure to carcinogens such as aflatoxin B1 are risk factors for HCC *(2)*.

Hepatoblastoma (HB) is another type of liver malignancy. Different from HCC, it is an embryonal tumor derived from undifferentiated embryonal tissue. Although its etiology remains unclear, it usually occurs in childhood with an annual incidence of 0.5–1.5 diagnoses per 1 million children younger than 15 yr of age in Western countries *(3)*. The incidence rate of HB is higher in patients with the Beckwith-Wiedemann syndrome or familial adenomatous polyposis *(4,5)*.

Cancer cell lines are commonly used as in vitro models for various types of cancer studies, including studies of carcinogenesis, potential antitumor drugs, and biomarker discovery. Advantages of using cell line models are that they are homogenous and their supplies are virtually unlimited. HepG2 is an HB cell line, derived from the HB tissue of a 15-yr-old male Caucasian. It has been frequently used as an in vitro cell line model in HCC-related studies, resulting in >500 reports *(6)* and occasionally has been wrongly regarded as an HCC cell line.

The present study aimed to compare the protein expression patterns between HepG2 cells and three well-characterized HCC cell lines derived from HCC tumor tissues. The findings of this study could help in understanding the differences in the cellular properties between HCC and HB and in answering whether HepG2 is an appropriate model for studies of HCC.

Materials and Methods

Cell Cultures

The HepG2 cells were obtained from American Type Culture Collection (ATCC) and cultured as recommended. The HCC cell lines used in this study are the three cell lines (HKCI-2, HKCI-3, HKCI-4) previously established by us *(7)*. They were cultured as previously described *(7)*. All three HCC cell lines were derived from the HCC tissues of patients with chronic HBV infection and/or liver cirrhosis. HKCI-4 cells expressed HBV core gene product. Immunohistochemical staining showed that all three HCC cells were of epithelial origin. Clinicopathological data of the two types of cell lines are listed in Table 1.

Sample Preparations

The HepG2 and HCC cells were cultured to about 60–70% confluence. The cells were

Table 1
Clinicopathologic Data in Patients, Primary Liver Cancers, and the Corresponding Cell Lines

Cell lines	Cirrhosis[a] (patient liver)	Serological viral status (patient serum)	HBV core gene[b] (primary tumor)	HBV surface gene[b] (primary tumor)	HBV core gene[b] (cell line)	HBV surface gene[b] (cell line)
HepG2	NA	NA	NA	NA	−	−
HKCI-2	+	−	−	−	−	−
HKCI-3	−	HBV	+	−	−	−
HKCI-4	+	HBV	+	+	+	−

Information about hepatocellular carcinoma (HCC) cell lines was as published (7). Information about HepG2 cells is available from American Type Culture Collection. NA, not available.

[a]Cirrhosis = the presence of underlying liver cirrhosis.

[b]Hepatitis B virus (HBV) status for HCC cell lines was confirmed by nested PCR analysis for the presence of HBV core and surface genes 7.

washed twice with Hanks buffer supplemented with 20 m*M* HEPES (pH 7.0) and once with 10 m*M* Tris-Cl (pH 7.0) containing 250 m*M* sucrose. The cells were lysed in cell lysis buffer (8 *M* urea, 40 mg/mL CHAPS, 40 m*M* Tris, and 65 m*M* dithiothreitol [DTT]) with the use of a sample grinding kit (Amersham Biosciences, Piscataway, NJ) and a hand-held motor. The protein concentrations of each sample were quantified with the two-dimensional gel electrophoresis (2-DE) quantification kit (Amersham). All cell lysates were stored at −20°C until use.

Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE)

The cell lysates were subjected to isoelectric focusing (IEF; the first dimensional separation) on a PROTEAN IEF cell (Bio-Rad, Hercules, CA). Eighty micrograms of total cellular protein of each cell lysate was mixed with IEF rehydration buffer (Bio-Rad) to a final volume of 300 μL. A 17-cm immobilized pH 3.0–10.0 nonlinear gradient IPG immobilin strip was passively rehydrated with the mixture for 16 h. IEF was carried at 20°C under programmed stepwise conditions: 100 V, 50 Vh; 200 V, 200 Vh; 500 V, 500 Vh; 1000 V, 1000 Vh; 8000 V, 24,000 Vh. After IEF, the IPG strip was washed briefly with water and equilibrated for 15 min in 6 *M* urea, 0.375 *M* Tris-Cl at pH 8.0, 0.02 g/mL SDS, 20% glycerol (v/v), and 1 mg/mL DTT. Then the strip was equilibrated in 6 *M* urea, 0.375 *M* Tris-Cl at pH 8.0, and 20% glycerol with 40 mg/mL iodoacetamide (IAA; Sigma, St. Louis, MO). Finally, the strip was equilibrated in 6 *M* urea, 0.375 *M* Tris-Cl at pH 8.0, 0.02 g/mL SDS, and 20% glycerol (v/v) for 10 min. For the second dimension separation, 8–16% gradient gel (Bio-Rad) was used. SDS-PAGE was performed on the Protean XL system (Bio-Rad). The gels were stained with the Plus-one silver staining kit (Amersham) according to the manufacturer's instructions. Developing time was controlled to avoid saturation. Each sample was analyzed at least twice.

Image Acquisition and Data Analysis

The stained gels were scanned with a densitometer GS-700 (Bio-Rad) as raw 2-DE images. After scanning, all the gels were stored at 4°C before further analysis. The gel images were processed and compared using the PDQuest software (version 7.11, Bio-Rad). On each gel image, the intensity of each spot was normalized against the total intensity of all valid spots. Protein quantities of twofold up- or downregulation between the two groups

were considered as differentially expressed spots.

Protein Identification by Peptide Mass Fingerprinting

The protein spots of interests were excised from the gels and placed into Eppendorf tubes. The gel pieces were first washed with milli-Q water, destained with a freshly prepared mixture of 1:1100 m*M* sodium thiosulfate and 30 m*M* potassium ferricyanide (III). Then the gel pieces were sequentially washed with milli-Q water, 50% methanol, 10% acetic acid, and 25 m*M* NH_4HCO_3. Afterward, the proteins in the gels were reduced with 10 m*M* DTT and subsequently alkylated with 50 m*M* IAA in 25 m*M* NH_4HCO_3. The gel pieces were washed again with 25 m*M* NH_4HCO_3, dehydrated with 25 m*M* NH_4HCO_3 in 80:20 acetonitrile (ACN)/water. After drying, the gel pieces were rehydrated with digestion buffer (0.1 mg/mL n-octylglucoside in 25 m*M* NH_4HCO_3, pH 8.0) containing 50 ng of sequencing grade trypsin (Sigma). Digestion was carried out at 37°C overnight. Digested peptides were extracted twice with 70:30 ACN/water containing 0.1% trifluoroacetic acid (v/v). The recovered peptides were spotted on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) sample plate and overlaid with α-cyano-4-hydroxycinnamic acid as matrix. All the samples were detected with the Voyager DE™-Pro MALDI-TOF MS system (Applied Biosystems, Foster City, CA). The acquired masses were externally calibrated with peptide mass standards (Applied Biosystems) and internally calibrated with trypsin autolyzed peptides. The acquired masses were submitted to PROFOUND (www.prowl.rockerfeller.edu/cgi-bin/ProFound) for protein database matching. A protein identification was considered successful when the PROFOUND probability score was 1.0 and the estimated *Z* score was higher than 1.65 *(8)*.

Results

Figure 1 shows the representative gel images of HepG2, HKCI-2, HKCI-3, and HKCI-4, respectively. On average, about 1300 protein spots from each cell line were detected. Compared with the HepG2 cells, the normalized quantities of 49 and 58 protein spots were found to be at least twofold higher and twofold lower, respectively, in all three HBV-associated HCC cell lines.

The differentially expressed spots were excised from the gels and subjected to peptide mass fingerprinting analysis. Protein identities of 30 protein spots were successfully obtained. The protein identities of the differentially expressed proteins and their relative abundances are summarized in Table 2. For those proteins with isoforms, the relative abundances of individual isoforms are also listed. Our experimental molecular weight/isoelectric point (M_r/p*I*) values of the identified proteins matched with theoretical M_r/p*I* values and the experimental M_r/p*I* values reported by Seow et al. *(9)*.

Figure 2 shows a zoom-view of the gel images. Isoforms of GTP:ATP phosphotransferase, and dehydrogenase/reductase SDR family member 2, and GTP-binding nuclear protein RAN were upregulated in HepG2, whereas GTP-binding nuclear protein RAN, phosphoglycerate mutase isozyme B, glyceraldehyde 3-phosphate dehydrogenase, transgelin 2, and isoforms of annexin II were upregulated in HCC cell lines. The differentially expressed proteins can be categorized as structural proteins, heat shock proteins (HSPs), metabolic enzymes, and transcription factors (Table 3).

Discussion

Conventional cytogenetic studies, comparative genomic hybridization studies, and PCR-based microsatellite studies showed significant differences in the chromosomal changes between HB and HCC *(24)*. HBs are characterized by a low number of chromosomal changes,

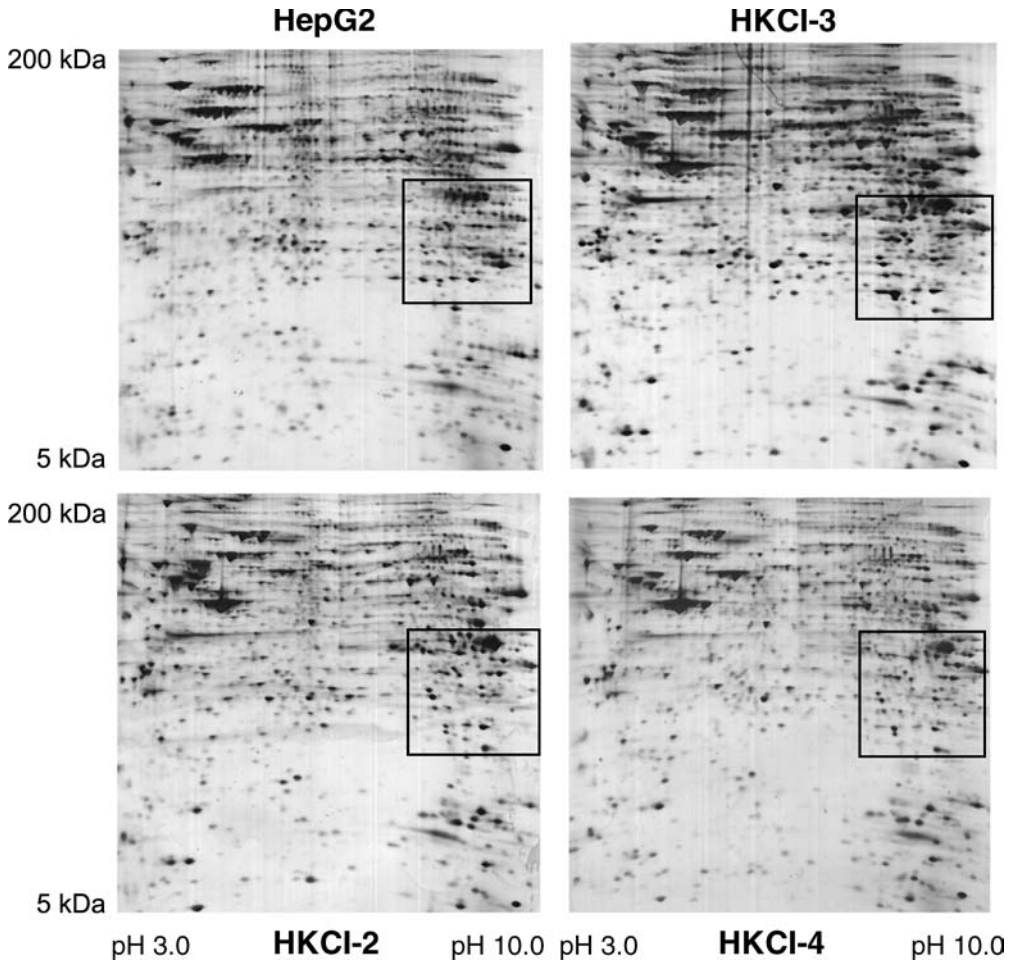

Fig. 1. Representative gel images of HepG2 and hepatocellular carcinoma cell lines. The boxes indicate the positions of zoom view images of the gels as shown in Fig. 2.

whereas HCCs have multiple chromosomal abnormalities. Our recent spectral karyotyping study also revealed that the chromosomal abnormalities that commonly occurred in HCCs did not present in HepG2 cells *(25)*. In this study, we also found significant differences in the expression levels of various proteins between HepG2 cells and HCC cells: those involved in various cellular functions including homeostasis, metabolism, cellular structure, gene transcription regulation, and apoptosis.

We used 2D-PAGE coupled with silver staining to find out the differences between HCC and HB. Although silver staining may not be the best choice for quantitative experiments, it is widely accepted for semiquantitative analysis. Silver staining has a linear dynamic range of >50-fold *(26)*. After normalization, the coefficients of variation of the quantities of the silver-stained protein spots are between 15 and 25% *(27,28)*, comparable to the values obtained by SYPRO Rudy staining.

We carefully reviewed previous studies on gene/protein expression patterns of HCC and liver cells/tissues. Some of these differentially expressed proteins had been identified in other HCC cell lines, hepatocytes, and serum/tissues

Table 2
Summary of the Profound Matching Scores and the Relative Abundance Ratio of the Differentially Expressed Proteins in HepG2 and HCC Cell Lines

SWISS-PROT accession number	SWISS-PROT entry name	Spot number	Protein description	Profound probability	Profound Est'd Z	Abundance ratio HCC: HepG2			% Sequence coverage	Experimental Theoretical M^r/pI	Experimental M^r/pI	M^r/pI from ref. *9*
						HKCI2	HKCI3	HKCI4				
P08865	RSP 4_HUMAN	0426	**34/67-kDa Laminin receptor**	1.0	2.09	2.20	2.50	2.06	26	32.8/4.8	41.6/4.8	NA
P10809	CH 60_HUMAN	1624	**60-kDa Heat shock protein, mitochondrial**	1.0	2.27				19	61.4/5.7	62.5/6.0	NA
			Acidic isoform			0.61	0.91	0.90				
			Basic isoform			0.28	0.45	0.42				
			Total expression ratio			0.40	0.74	0.63				
P38646	GR 75_HUMAN	2730	**Stress-70 protein, mitochondrial**	1.0	2.35				23	73.8/6.0	74.4/6.5	76.7/5.6
			Basic isoform			0.29	0.25	0.07				
			Total expression ratio			0.63	0.60	0.76				
P04792	HS 27_HUMAN	4226	**HSP 27 Stress-responsive protein**	1.0	2.41	Not detected in HepG2			42	22.3/8.1	28.2/6.0	27.3/6.0
P06733	EN OA_HUMAN		**Alpha enolase**									
		3413	Isoform 1	1.0	1.83	0.10	0.01	0.01	26	47.2/7.0	43.1/5.6	50.2/6.6
		3424	Isoform 2	1.0	2.30	0.50	0.37	0.60	23		43.2/5.7	50.2/6.7
		4404	Isoform 3	1.0	2.40	4.40	3.01	4.51	27		43.4/5.8	50.1/6.9
		4505	Isoform 4	1.0	2.37	0.03	0.13	0.10	30		50.3/5.8	49.8/7.1
		4527	Isoform 5	1.0	2.24	0.17	0.50	0.09	22		52.6/6.0	50.1/7.0
			Total expression ratio			0.78	0.72	0.77				
P30837	DH A5_HUMAN	4603	**Aldehyde dehydrogenase, mitochondrial (precursor)**	1.0	1.79	0.18	0.24	0.16	15	57.2/6.4	57.7/5.8	NA
P18669	PM G1_HUMAN	6214	**Phosphoglycerate mutase isozyme B**	1.0	2.27	2.50	1.70	2.00	36	28.8/6.7	33.8/4.7	27.6/6.2

Q92945	FUB2_HUMAN	6829	Far upstream element binding protein 2	1.0	2.37	Not detected in HepG2			37	73.1/6.9	77.9/6.8	NA
P17080	RAN_HUMAN	7411	**GTP-binding nuclear protein RAN**	1.0	2.38	4.06	5.64	5.41	44	24.3/6.6	23.8/6.9	23.4/6.9
P30086	PEBP_HUMAN	7431	**Phosphatidyl-ethanolamine-binding protein (neuropeptide h3)**	1.0	2.41	0.23	0.10	0.24	48	20.9/7.5	20.7/7.1	NA
P07355	ANEX_HUMAN		**Annexin II**									
		7306	Isoform 1	1.0	2.40	8.80	6.26	20.76	28	38.6/7.7	36.3/6.9	35.7/7.1
		7324	Isoform 2	1.0	2.38	23.21	25.48	28.60	51		36.3/7.0	37.1/6.7
			Total expression ratio			17.40	17.74	25.45				
P22570	ANDRO_HUMAN		**NAPDH: adrenodoxin oxidoreductase, mitochondrial (precursor)**									NA
		7525	Isoform 1	1.0	2.39	0.05	0.10	0.10	43	53.8/9.1	52.2/8.0	
		7530	Isoform 2	1.0	2.23	0.03	0.06	0.06	47		51.8/8.1	
			Total expression ratio			0.04	0.08	0.08				
P14618	KPY1_HUMAN	7620	**Pyruvate kinase**	1.0	2.34	2.46	1.78	2.40	18	57.9/7.8	63.2/7.0	55.5/7.2
Q13268	DHS2_HUMAN		**Dehydrogenase/ reductase SDR family member 2**									NA
		8137	Isoform 1	1.0	1.90	0.05	0.05	0.08	18	27.4/9.5	26.6/9.2	
		8239	Isoform 2	1.0	2.34	0.02	0.02	0.08	44	27.4/9.5	28.2/9.1	
			Total expression ratio			0.04	0.04	0.08				
P37802	TAG2_HUMAN		**Transgelin2**									NA
		8142	Isoform 1	1.0	2.36	Not detected in HepG2			14	22.8/8.6	21.9/7.6	
		8144	Isoform 2	1.0	2.41				58		22.0/7.6	
			Total expression ratio									
P21796	POR1_HUMAN	8237	**Voltage-dependent anion-selective channel protein 1**	1.0	2.37	3.08	3.07	3.05	44	30.8/8.8	32.7/8.2	31.0/8.4
P16152	DHCA_HUMAN	8335	**Carbonyl reductase (NADPH) 1**	1.0	2.35	Not detected in HepG2			29	30.4/8.9	33.9/7.5	NA

continued

Table 2 (*continued*)

SWISS-PROT accession number	SWISS-PROT entry name	Spot number	Protein description	Profound probability	Profound Est'd Z	Abundance ratio HCC:HepG2			% Sequence coverage	Experimental Theoretical M^r/p*I*	Experimental M^r/p*I*	M^r/p*I* from ref. *9*
						HKCI2	HKCI3	HKCI4				
P04406	G3P2_HUMAN	8337	**Glyceraldehyde 3-phosphate dehydrogenase, liver**	1.0	2.33	3.52	8.36	8.53	36	36.0/8.7	39.1/7.9	37.4/7.8
P04075	ALFA_HUMAN	8418	**Fructose-bisphosphate aldolase A**	1.0	2.17	3.80	4.31	1.70	18	39.4/8.7	47.0/7.6	43.0/7.8
Q14588	Z234_HUMAN	8514	**Zinc finger protein 234 (fragment)**	1.0	2.37	12.35	23.16	2.56	33	80.3/8.9	54.1/7.9	NA
Q7Z531	Q7Z531		**GTP:ATP phosphotransferase**									NA
		9213	Isoform 1	1.0	2.06	0.01	0.07	0.01	49	24.3/8.7	28.0/8.7	
		9220	Isoform 2	1.0	2.28	0.03	0.07	0.38	47		27.9/9.0	
			Total expression ratio			0.08	0.07	0.26				

The calculation of relative abundance ratios was based on normalized values. HCC, hepatocellular carcinoma; NA, no information available.

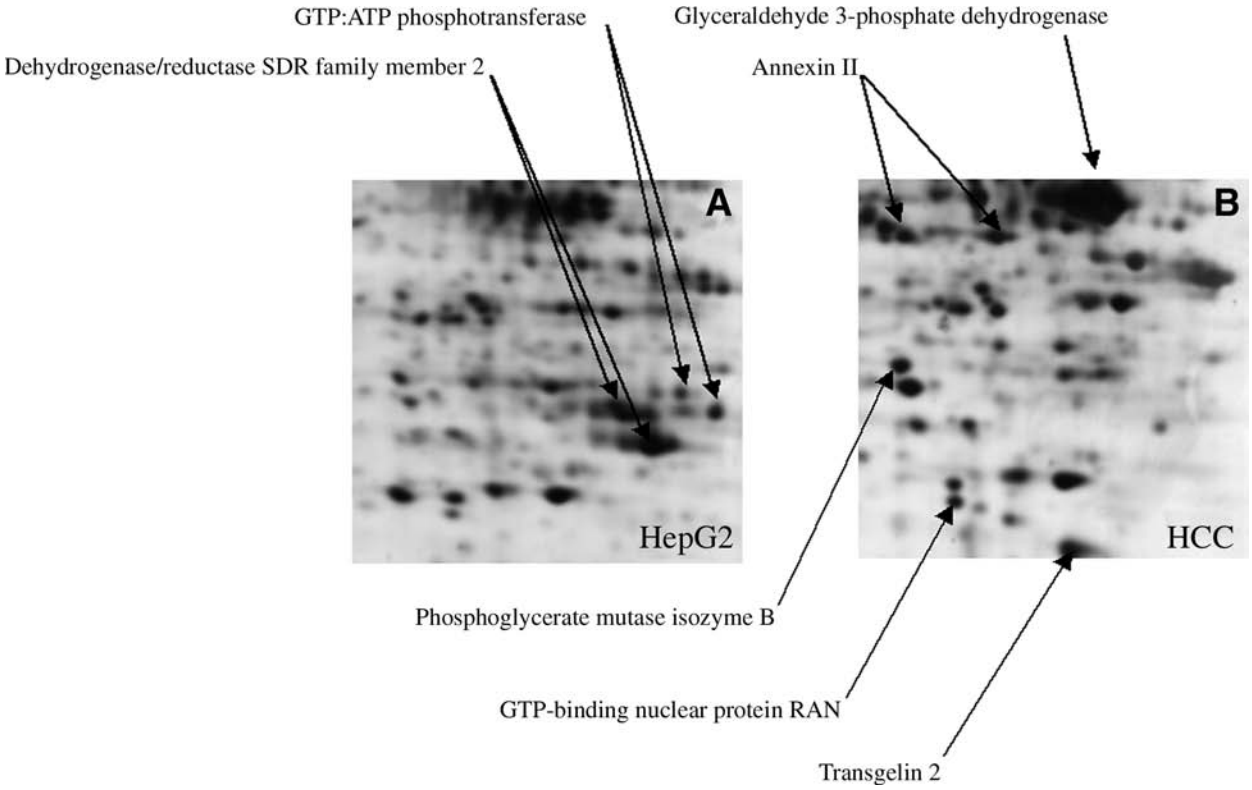

Fig. 2. Zoom views of a representative region in gel images showing differential expressions of the proteins in HepG2 cells and hepatocellular carcinoma (HCC) cells. Arrows indicate the proteins that had twofold higher expression in HepG2 **(A)** and HCC **(B)** cell lines.

of HCC patients. The expression patterns of these proteins in liver, other cell lines, and tissues are listed in Table 3. Our findings are consistent with those of other investigators; for example, in our study, HSP27 is highly expressed in HCC cell lines but not in HepG2 cells. This protein is also highly expressed in an HBV-associated HCC-M cell line and in a BEL-7404 HCC cell line *(11,12,14)*. HepG2 expressed a high level of HSP27 only when it was stably transfected with hepatitis B viral X protein. Laminin receptor is highly expressed in the HCC cell lines examined but not in HepG2 cells. This protein is also highly expressed in an HCC-M cell line *(11)* but is not found in HepG2 cell membrane *(19)*. Another example is that dehydrogenase/reductase is highly expressed in HepG2 cells but not in HCC cell lines in our study. This protein was reported to be expressed in HepG2 *(22)*.

Table 4 shows the biological roles of the differentially expressed proteins related to HBV and carcinogenesis. The HSPs were differentially expressed between the HepG2 and the HCC cell lines. HSPs are a group of highly conserved proteins; they can be classified according to their molecular masses into four major families: small, low-molecular-weight HSP (sHSP), HSP60, HSP70, and HSP90. They are synthesized by cells and tissues in response to heat and various stress conditions including carcinogenesis. HSPs are usually highly expressed in many types of tumor. HSPs act as molecular chaperones; they can protect proteins from degradation and enhance protein refolding, thus inhibiting apoptosis and enhancing cell survival under stress

Table 3
Expression Patterns of the Differentially Expressed Proteins in Other HCC Cell Lines, Hepatoblastoma Cell Lines, Differentiating Hepatocytes, and Serum

Protein name	Ratio of expression (HCC:HepG2)[a]	Reported expression patterns in HCC, hepatoblastoma cell lines, differentiating hepatocytes, and serum
Heat shock proteins		
HSP 27 stress-responsive protein	Higher	↑ in HepG2 cells transfected with hepatitis B viral X protein *(10)* Expresses in HCC-M[b] cell line *(11,12)* Differentially expresses between normal liver and HCC tumor tissues *(13)* Differentially expresses between normal liver cell line (L-02) and hepatoma cell line (BEL-7404) *(14)*
60-kDa heat shock protein	Lower	Expresses in HepG2 *(15)* and HCC-M *(11)*, liver tissue *(13)* Highly expressed in a variety of carcinoma *(16)*
Stress-70 protein	Lower	↑ in HBV-associated HCC *(17)* and early HCV-associated HCC *(18)* Expresses in HCC-M cell line *(11)* and liver tissue *(13)*
Structural proteins		
34/67-kDa Laminin receptor	Higher	Expresses in HCC-M cell line *(11)*. HepG2 cells do not express membrane laminin receptor *(19)*.
Annexin II (include all detected isoforms)	Higher	Expresses in HCC-M cell line *(11,20)*
Transgelin 2 (include all detected isoforms)	Higher	Expresses in HCC-M cell line *(11)* and normal liver *(13)*
Voltage-dependent anion-selective channel protein 1	Higher	Expresses in HCC-M cell line *(11,20)*
Enzymes		
Alpha enolase (include all detected isoforms)	Lower	Expresses in HCC-M *(21)* and liver tissue *(13)*
Aldehyde dehydrogenase	Lower	Detected in normal liver tissue *(13)* and expresses in HCC-M cell line *(11)*
Phosphoglycerate mutase isozyme B	Higher	Expresses in HCC-M cell line *(11,20)* and normal liver tissue *(13)*
Phosphatidylethanolamine-binding protein	Lower	Expresses in HCC-M cell line *(11)*
NADPH:adrenodoxin oxidoreductase	Lower	NA
Pyruvate kinase	Higher	Expresses in HCC-M cell line *(11,20)*. ↑ in HCC serum *(21)*

Dehydrogenase/reductase SDR family member 2	Lower	Expresses in HepG2 *(22)*
Carbonyl reductase (NADPH) 1	Higher	Expresses in normal liver tissue *(13)*. ↓ expression in HCC samples *(23)*
Glyceraldehyde 3-phosphate dehydrogenase, liver	Higher	Expresses in HCC-M cell line *(11,20)* and normal liver tissue *(13)*
Fructose-bisphosphate aldolase A	Higher	Expresses in HCC-M cell line *(11,20)*
GTP:ATP phosphotransferase (include all detected isoforms)	Lower	NA
Transcriptional factors		
GTP-binding nuclear protein RAN	Higher	Expresses in HCC-M cell line *(11,20)*
Zinc finger protein 234 (Fragment)	Higher	NA
Far upstream element binding protein 2	Higher	Expresses in HCC-M cell line *(11)*

[a]The ratio of the normalized spot intensities of hepatocellular carcinoma (HCC) and HepG2 cell lines. "Higher" indicates that the HCC cell line expression level of the protein spot is higher than that in HepG2 cells, whereas "lower" indicates that the protein expression level of the protein spot is lower when compared to that in HepG2 cells.

[b]HCC-M cell line is hepatitis B virus (HBV) associated.

NA, no information available; HCV, hepatitis C virus; ↑, increased;↓, decreased.

Table 4
Biological Roles of the Differential Expressed Proteins Related to Carcinogenesis

Protein name	Biological roles associates with HBV and/or carcinogenesis					
	Drug resistance	Cell growth	Apoptosis	Cell differentiation	Metastasis	Remarks
Heat shock proteins						
HSP 27 stress-responsive protein	Related to multidrug resistance *(29–31)*	–	Interacts with key components of the apoptotic signaling pathway *(32)*, ↑ inhibits apoptosis *(33)*	–	↑ associated with shorter survival *(34)* and increased invasiveness *(35)*	–
60-kDa heat shock protein	Anticancer drugs stimulate its expression *(36)*	–	Involved in the formation of pre-apoptotic complex *(37)*	–	–	Essential for in vivo HBV replication *(15)*
Stress-70 protein	Involved in drug sensitivity *(36)*, ↑ in cisplatin resistance carcinoma cell lines *(38)*, ↑ in heat-induced drug resistance carcinoma cell lines *(39)*	–	Binds to apoptosis-inducing factor (40), ↑ inhibits apoptosis *(33)*	–	–	Expresses in cellular cytoplasm and cell surface - of HBV associated HCC *(41)*. May improve cell survival by protecting proteins from degradation and enhancing refolding refolding *(42,43)*
Structural proteins						
34/67-kDa laminin receptor	A multidrug resistance-associated protein *(44)*	–	–	↑ induces differentiation *(45)*	↑ increases invasiveness in HBV-associated HCC *(32)*	–

Annexin II	Associated with multidrug resistance in colorectal cancer and small cell lung cancer cell line (H630-R10) *(46,47)*	Involved in DNA synthesis and cell proliferation *(48)*	Involved in apoptosis *(49)*	–	↑ inhibits prostate and lung carcinoma cell migration *(50)*	–
Transgelin 2	–	–	–	–	–	↓ during breast and colon cancer development *(51)*
Voltage-dependent anion-selective channel protein 1	–	–	Bcl-2 and induces apoptosis *(52)*	–	–	
Enzymes						
Alpha enolase	Related to cisplatin resistance property in head and neck carcinoma cell line *(38)*	–	–	–	–	Downregulates *c-myc* oncogene expression *(53)*
Aldehyde dehydrogenase	Related to multidrug resistance (38,54)	–	–	–	–	Polymorphisms modify the risk of development of HCV and cirrhosis-associated HCC *(55)* but not related to HBV-associated HCC *(56)*
Phosphoglycerate mutase isozyme B	–	–	–	–	–	↑ of activity observed in breast carcinoma *(57)* and lung, colon, and liver carcinoma *(58)*, but ↓ in brain carcinoma *(59)*

continued

Table 4 (*continued*)

Biological roles associates with HBV and/or carcinogenesis						
Protein name	Drug resistance	Cell growth	Apoptosis	Cell differentiation	Metastasis	Remarks
Phosphatidylethanolamine-binding protein	–	↑ inhibits cell proliferation and differentiation *(60)*	–	–	–	–
Pyruvate kinase	↓ in drug resistance cell line *(61)*	–	–	–	–	–
NADPH:adrenodoxin oxidoreductase	Involved in drug metabolism *(62)*	–	–	–	–	–
Dehydrogenase/reductase SDR family member 2	–	Synthesis in growth arrest HepG2 cell *(63,64)*	–	–	–	–
Glyceraldehyde 3-phosphate dehydrogenase, liver	–	–	–	–	–	mRNA usually higher expressed in HCC (65,66)
Fructose-bisphosphate aldolase A	–	–	–	–	–	10-fold higher in patients with HCC than in chronic hepatitis C or cirrhosis *(67)*
Transcriptional factors						
Far upstream element binding protein 2	–	↑ reduces cell proliferation *(68)*	–	–	–	–
GTP-binding nuclear protein RAN	–	↑ reduces cell proliferation and decreases total cell numbers *(69)*	–	Associates with cell differentiation in B-cells *(70)*	–	–

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; ↑, increase; ↓, decrease.

conditions such as a drug challenge *(71)*. HSPs are differentially expressed between the HCC cell lines and the HepG2 cell line. Thus, their properties in drug resistance, apoptosis, and invasiveness may be different.

For structural proteins, laminin receptor and annexin II are upregulated in HCC cells, and these two proteins were also expressed in HCC-M cell lines *(11,20)*. There is much evidence to indicate that the 67-kDa laminin receptor is important in the progression of a wide variety of carcinomas *(72)*. Annexins are Ca^{2+} and phospholipid binding proteins that have been implicated in the regulation of exocytic and endocytic pathways. Expression of annexin II was demonstrated in HCC and was not detected in normal liver extracts *(73)*. Both laminin receptor and annexin II are associated with drug-resistance properties (Table 4). Laminin receptor is HBV associated; its upregulation is a signal of increasing invasiveness in HBV-associated HCC *(74)*. Laminin receptor is highly expressed in the HCC-M cell *(11)* and also in our HCC cells, whereas Zheng et al. *(19)* demonstrated very low expression levels of membrane laminin receptor in HepG2 cells. Moreover, annexin is also involved in apoptosis. Thus, the apoptotic and drug-resistance properties of HepG2 and HCC cell lines should be different.

Expression levels of metabolic enzymes are commonly altered in carcinoma tissues. Several enzymes involved in the glycolytic pathway were upregulated in HCC cell lines. Aldose A, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, and pyruvate kinase were upregulated in HCC cell lines. This indicates that the cell lines may have different metabolic properties. In addition, α-enolase, aldehyde dehydrogenase, pyruvate kinase, and NADPH: adrenodoxin oxidoreductase are involved in drug metabolism. These differentially expressed proteins reflect the different properties of the cell lines.

Far upstream element binding protein 2 and GTP-binding nuclear protein RAN are upregulated in HCC cell lines. These two proteins are involved in transcription and growth regulation. Far upstream element binding protein 2 is also involved in cell differentiation *(70)*, thus indicating that the two types of cells are using different growth regulatory mechanisms.

In conclusion, proteins involved in regulating homeostasis, drug resistance, apoptosis, cell differentiation, cell growth, and metastasis were differentially expressed in the HCC cell lines and in HB cell line HepG2. Hence, HepG2 may not be a good cell line model for studying HCC. Our results also suggest that the cellular properties of HCC and HB are different.

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