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Review

Proteome database of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC or hepatoma) is the most common primary cancer of the liver. Persistent viral infection by the hepatic B or C virus is probably the most important cause of HCC worldwide. It is responsible for approximately one million deaths each year, predominantly in the underdeveloped and developing countries, but its incidence is also on the rise in the developed countries. For most patients suffering from HCC, long-term survival is rare, as they are presented late and are often unsuitable for curative treatment. Thus there is great interest to identify novel HCC diagnostic markers for early detection of the disease, and tumour specific associated proteins as potential therapeutic targets in the treatment of HCC. Proteome analyses of HCC cell lines and liver tumour tissues should facilitate the screening and discovery of these HCC proteins. The creation of a comprehensive HCC proteome database would be an important first step towards achieving this goal. This review presents an update of the two-dimensional electrophoresis proteome database of the cell line, HCC-M, which is also now freely accessible through the World Wide Web at http://proteome.btc.nus.edu.sg/hccm/.

Keywords: Reviews; Proteomics; Hepatocellular carcinoma

Contents

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1. Introduction

Proteomics refers to the study of the proteome, which is the total protein complement of a genome. There are two major proteomic approaches: one of which is concerned with the global analysis of the total cellular proteins in a given cell type or tissue (protein expression proteomics), while the other seeks to define protein–protein interactions to understand gene function (functional or cell mapping proteomics) [1]. Thus, it has recently been hailed as the next frontier in biology in the postgenomic era. There are numerous applications in proteomics, but the one that is most well established is in the clinical and biomedical fields [2,3]. For example, using protein expression proteomics, disease specific/associated proteins can be identified by comparing the protein profiles of normal versus diseased tissues or biological fluids. Since these proteins are potential diagnostic tools or leads for drugs, proteomics also has great potential in the modern drug discovery process [4]. The disease that has received the greatest attention by proteomics studies is cancer. Several excellent reviews have been published on this subject recently [1,5,6]. In this review, we present an update on the results of the proteome analysis of the hepatocellular carcinoma (HCC or hepatoma) cell line, HCC-M, that would also now be made available on the world wide web at http://proteome.btc.nus.edu.sg/hccm/.

2. Hepatocellular carcinoma

2.1. Epidemiology and aetiology

HCC is the most common primary cancer in liver, and is responsible for about 1 million deaths per year [7]. For most patients suffering from HCC, long-term survival is rare as they usually die within a year of diagnosis. HCC has been a malignancy of the underdeveloped and developing countries but its incidence is also on the rise in the developed countries. Depending on geographical location, HCC is four to eight times more common in males than in females, and its occurrence also increases progressively with age [7].

Persistent viral infection is probably the most important cause of HCC. Two viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV), cause almost all these tumours. For example, the risk of HCC in a chronic HBV carrier is increased 100-fold as compared to a non-infected individual [8]. HBV infection leads to chronic liver injury, and this includes inflammation, liver regeneration, liver fibrosis and cirrhosis. In fact, it has been shown that more than 80% of patients with HCC have a cirrhotic liver [7]. Other aetiological factors of HCC include exposure to aflatoxins, excessive alcohol consumption, haematochromatosis, tyrosinaemia, and Wilson's disease [7,8].

2.2. Treatment

The treatment options available to patients with HCC are surgery, systemic chemotherapy, loco-regional treatment, and symptomatic relief [8]. Of these, only surgery has the potential to cure. However, at presentation, liver resection is only feasible for 10–15% of patients. The reasons for this low resectability rate include extensive local disease, presence of extra-hepatic disease, and poor functional liver reserve precluding any form of liver resection. In the light of this, there is a need to develop better methods to detect HCC at an early stage to allow the performance of curative surgery. By analysing the proteome of HCC, one hopes to identify novel diagnostic markers and specific disease associated proteins that are potential therapeutic targets in the treatment of HCC [8].

3. Proteome analysis of hepatocellular carcinoma

Several hepatoma cell lines [9–11] have been used for proteome analyses with the view to better understand the underlying process of hepatocarcinogenesis. Cell lines were chosen as they were more homogeneous in comparison to liver tumour tissues. Moreover, cell lines derived from human tumours have been used extensively as in vitro models of various diseases. For example, in an earlier publication, Wirth et al. [9], on the basis of 60 commonly expressed human liver proteins, reported that the proteins present in the nontransformed cell lines, Chang and WRL-68, were similar to those found in normal human liver. However, proteins expressed in the human hepatoma derived cell lines, HepG2, FOCUS, Huh-7 and SK-Hep1 were markedly different from those found in normal liver [9]. In a more recent study, Yu et al. [11] also reported differences in the proteins expressed between a human hepatoma derived (BEL-7404) and normal (L-02) liver cell line using two-dimensional electrophoresis (2-DE) and liquid chromatography–ion-trap mass spectrometry.

The most comprehensive proteome analysis of a hepatoma cell line, HCC-M, was carried out recently by Seow et al. [12], Ou et al. [13], and Choong et al. [14]. An integrated approach consisting of 2-DE, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), nanoelectrospray ionisation tandem MS (nESI-MS–MS), bioinformatics, and molecular biology techniques was employed to separate, identify and characterise the expressed proteins of this cell line. These proteins have now been organised into an interactive protein database that integrates the spots with the 2-DE map, and will be posted on the world wide web.

We present below the brief experimental protocols used in this proteomics project, with emphasis on some of the newer techniques, such as sample loading and fluorescent staining with SYPRO Ruby, that were used since our original publication [12] and an update [13]. The results on these newer experiments and the web database will be presented and discussed.

3.1. Cell culture and sample preparation

The HCC-M cell line was cultured as described previously [12], in Dulbelcco's modified Eagle medium (DMEM) supplemented with 10% foetal calf serum (FCS), and harvested once a monolayer culture was attained. During harvesting, the cells were rinsed with DMEM without FCS, and the harvested cells were stored at −80 °C. The harvested HCC-M cells were disrupted with a cocktail of 7 *M* urea, 2 *M* thiourea, 4% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS), 40 m*M* Tris, 1 m*M* phenylmethylsulphonyl fluoride (PMSF), 50 μg/ml DNase I, and 50 μg/ml RNase A.

3.2. Isoelectric focusing

The first dimensional isoelectric focusing (IEF) experiment was carried out on precast 18 cm (or 13 cm) immobilised pH gradient (IPG) strips at 20 °C with a maximum current setting of 50 μA/strip in an IPGphor electrophoretic unit (Amersham Biosciences, Uppsala, Sweden). Two types of ceramic strip holders were used for IEF: the regular strip holders, and the newer cup-loading strip holders.

3.2.1. Regular strip holder

The strips were rehydrated at 30 V for 6 h and 60 V for a further 6 h in the regular strip holders in 350 μl (250 μl for 13 cm strips) of sample containing 7 *M* urea, 2 *M* thiourea, 4% CHAPS, 20 m*M* dithiothreitol (DTT), and 0.5% IPG buffer. The amount of protein loaded was ~120 μg. After rehydration, IEF was carried out according to the following conditions: (i) 200 V, 200 Vh; (ii) 500 V, 500 Vh; (iii) 1000 V, 500 Vh; (iv) 1000–8000 V gradient, 2250 Vh; and (v) 8000 V, 32 000 Vh (24 000 Vh for 13 cm strips). Voltage increases for (i–iii) were performed on a step-wise basis, while the increase for (iv) was on a linear gradient.

3.2.2. Cup-loading strip holder

The strips were rehydrated overnight in 340 μl of 7 *M* urea, 2 *M* thiourea, 4% CHAPS, 20 m*M* DTT, and 0.5% IPG buffer. After rehydration, 10 μl of sample was loaded onto the anodic end of the IPG strip using a loading cup. The amount of protein loaded was ~120 μg. IEF was performed according to the following regiment: (i) 200 V, 100 Vh; (ii) 500 V, 250 Vh; (iii) 1000 V, 500 Vh; (iv) 1000–8000 V gradient, 2250 Vh; and (v) 8000 V, 32 000 Vh. Again, voltage increases for (i–iii) were performed on a step-wise basis, while the increase for (iv) was on a linear gradient.

3.3. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis

Before carrying out the second-dimensional sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), the strips were subjected to a two-step equilibration process: the first being reduction with DTT, followed by a second alkylation step with iodoacetamide (IAA), as described previously [12]. SDS–PAGE was performed on 1.0 mm thick 10% polyacrylamide gels at 10 °C, either at a constant voltage of 110 V in an ISO-DALT (for both 13- and 18-cm strips) apparatus (Amersham Biosciences), or at a constant current of 30 mA per gel in a PROTEAN II xi Cell IPG Conversion (for 18-cm strips) or a PROTEAN II xi Cell (for 13-cm strips) unit (Bio-Rad, Hercules, CA, USA).

3.4. Visualisation

The protein spots on the 2-DE gels were visualised using two different staining methods: silver staining, and fluorescent staining.

3.4.1. Silver staining

Silver staining of the gels was performed as described previously [12]. Briefly, the gels were fixed in 50% methanol, 5% acetic acid in water for 30 min followed by washing in 50% methanol in water for 10 min. The gels were then washed again with water for 60 min and sensitised with 0.02% sodium thiosulphate for 2 min. After the gels were rinsed twice with water for 1 min each, they were incubated in chilled 0.1% silver nitrate for 40 min at 4 °C. After rinsing with two changes of water for 1 min each, the gels were developed in 0.04% formalin in 2% sodium carbonate. When the desired intensity was attained, the development was stopped with 1.5% EDTA for 10 min. The staining procedure was completed by three rinses with water for 5 min each.

3.4.2. Fluorescent staining

Fluorescent staining was carried using the preprepared SYPRO Ruby fluorescent dye from Molecular Probes (Eugene, OR, USA), according to the manufacturer's instruction. The 2-DE gels were fixed in 10% methanol, 7% acetic acid in water for 30 min, before being incubated in the dark with the SYPRO Ruby dye for at least 3 h. The gels were rinsed twice with water for 5 min each, before being scanned on the Typhoon 8600 Imager (Amersham Biosciences).

3.5. Reduction and alkylation

After the protein spots were excised manually as described previously [12], they were subjected to a reduction and alkylation step before proteolysis. In essence, each excised spot was soaked with 150 μl of washing solution consisting of 2.5 m*M* ammonium bicarbonate in 50% aqueous acetonitrile (ACN), and stored at 4 °C for at least 24 h. A fresh aliquot of washing solution was replaced and each spot was incubated for 20 min at 37 °C, followed by drying in a centrifugal concentrator. The spots were then subjected to reduction and alkylation as de-

scribed [15]. Briefly, 20 μl of 10 m*M* DTT in 100 m*M* ammonium bicarbonate was added to each gel spot and incubated at 56 °C for 1 h. After cooling to room temperature, each spot was then incubated with 20 μl of 55 mM IAA in 100 m*M* ammonium bicarbonate in the dark at ambient temperature for 45 min. After washing each spot with 100 μl of 100 m*M* ammonium bicarbonate for 10 min, the gel spots were dehydrated with 100 μl of ACN for 10 min. The washing and dehydration steps were repeated, before the spots were dried in a centrifugal concentrator.

3.6. Enzymatic digestion

Enzymatic digestion was performed with the addition of 10 μl of 0.02 μg/μl modified trypsin in 25 m*M* ammonium bicarbonate to each gel spot, and incubated at 37 °C for 16 h with shaking. To enhance peptide extraction, 10 μl of 0.1% trifluoroacetic acid (TFA) in 50% aqueous ACN was added to each spot after the tryptic digestion, and sonicated for 20 min.

3.7. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry

MALDI-TOF MS analyses were performed as described previously [12]. Essentially, 1 μl of the extracted sample from each of the gel spots was dispensed onto the MALDI sample plate with 1 μl of matrix solution (10 mg/ml α-cyano-4-hydroxycinnamic acid, 0.1% TFA, 50% ACN), and allowed to dry under ambient conditions. The acquisition of spectra for each sample was performed using the delayed extraction and reflector mode as described [12]. Spectra were automatically calibrated upon acquisition using a two-point calibration with residual porcine trypsin autolytic fragments (842.51 and 2210.10 $[M+H^+]$ ions). Assignment of peaks and protein identification were performed automatically using the AutoMS-Fit software, which is part of the Proteomics Solution 1 system (Applied Biosystems, Foster City, CA, USA). Searches were queried against the SWISS-PROT and NCBI non-redundant databases, using parameters described previously [12].

3.8. Nanoelectrospray ionisation tandem mass spectrometry

Samples that did not return any confident matches from the MALDI-TOF MS database searches were subjected to nESI-MS–MS analysis as described [13]. Briefly, the remaining tryptic digested protein samples were each passed through a C_{18} ZipTip (Millipore, Bedford, MA, USA), and eluted with 2 μl of 1% formic acid in 60% methanol. Each eluted sample was loaded into a spray capillary needle and the spray was initiated by applying a potential of 850 V. Data acquisition, spectra processing, and database searches were performed using the Analyst QS software (Applied Biosystems). The searches were performed either manually against the SWISS-PROT and NCBI non-redundant databases, or automatically using the Mascot search engine (Matrix Science, London, UK) [16].

3.9. Two-dimensional electrophoresis maps

With the advent of high-resolution and reproducible 2-DE using IPG strips in the first dimension, it is now feasible to obtain high quality 2-DE maps of tissues and cells with reasonable speed for proteome analyses. We present here the 2-DE maps of seven hepatoma derived cell lines, HCC-M, Hep3B, HepG2, SK-Hep1, Huh-4, Huh-7, and PLC/PRF/5, and a non-transformed cell line, Chang liver (Fig. 1). It is apparent that these 2-DE maps exhibited differences in the protein profiles when compared with each other, and hence can be used as a basis to classify or differentiate the various hepatoma cell lines. This result is consistent with the recent gene expression profile studies of Kawai et al. [17], who showed that the α-fetoprotein producing cell lines, HepG2, Huh-7, Hep3B, PLC/PRF/5 and Huh-6 have common gene-expression profiles when compared with HLE and SK-Hep1, which are α-fetoprotein negative hepatoma cell lines, and cancer cell lines of non-hepatocyte origin (HeLa and KMBC). In addition, HepG2, Huh-7, and Hep3B which had higher expressions of α-fetoprotein shared a common gene expression profile when compared with the other α-fetoprotein producing cells (Huh-6 and PLC/PRF/5).

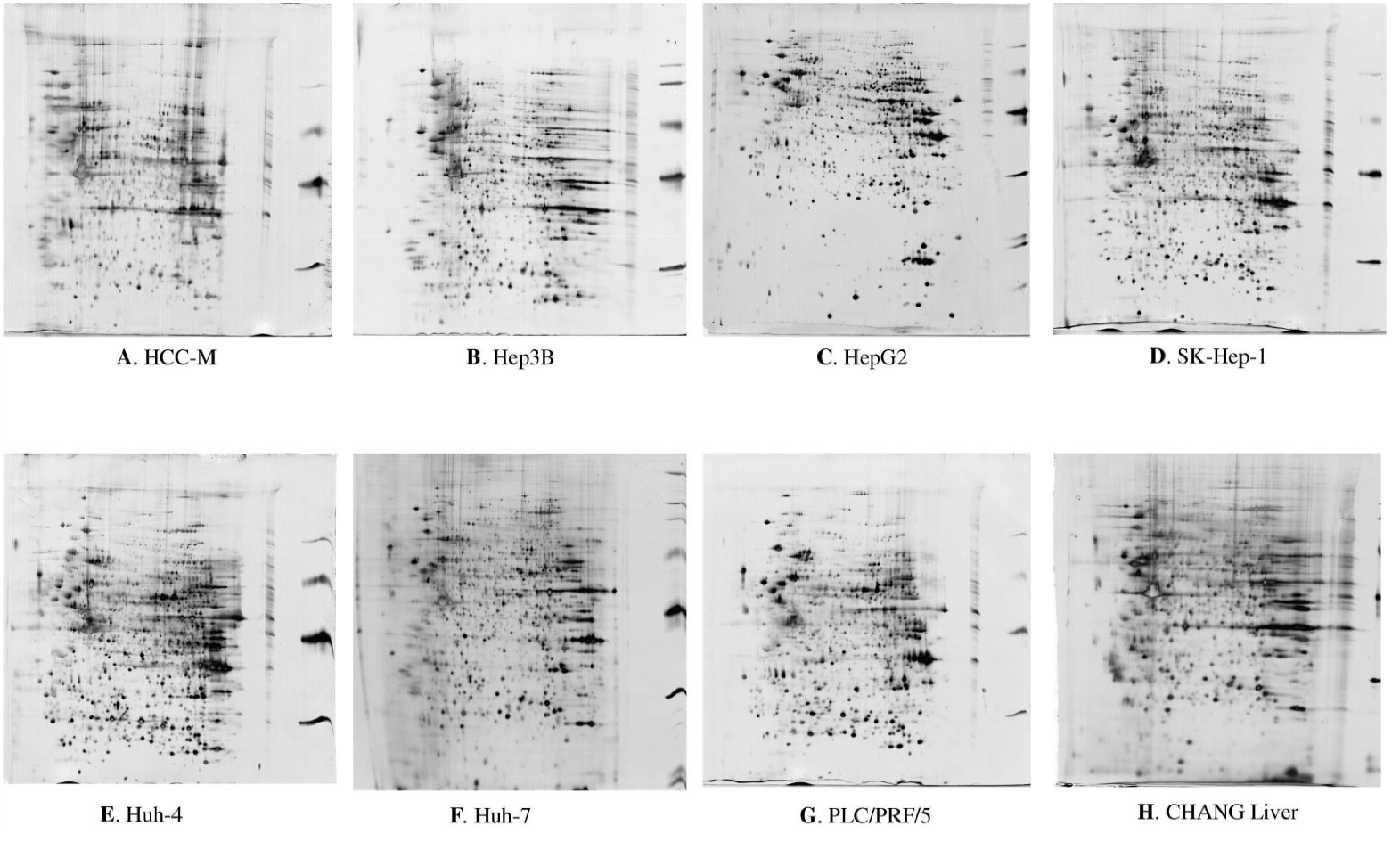

Fig. 1. 2-DE maps of human HCC cell lines. Cell lysate proteins were first separated on 13 cm Immobiline DryStrips pH 3–10 NL, using regular strip holders. The proteins were then separated using 10%T SDS–polyacrylamide gels using either the ISO-DALT or PROTEAN II xi cell electrophoretic tanks at 110 V and 30 mA/gel, respectively. The gels were silver stained. Protein loading was ~120 μg/gel.

3.10. Cup-loading versus in-gel rehydration sample application

It is a well-known fact that using the in-gel rehydration method for sample application on IPG strips, the separation of alkaline proteins was not satisfactory. The result is the presence of horizontal streaks in the SDS–polyacrylamide gel towards the basic end of the IPG strip, and there are a number of reasons for this phenomenon [18]. We have found

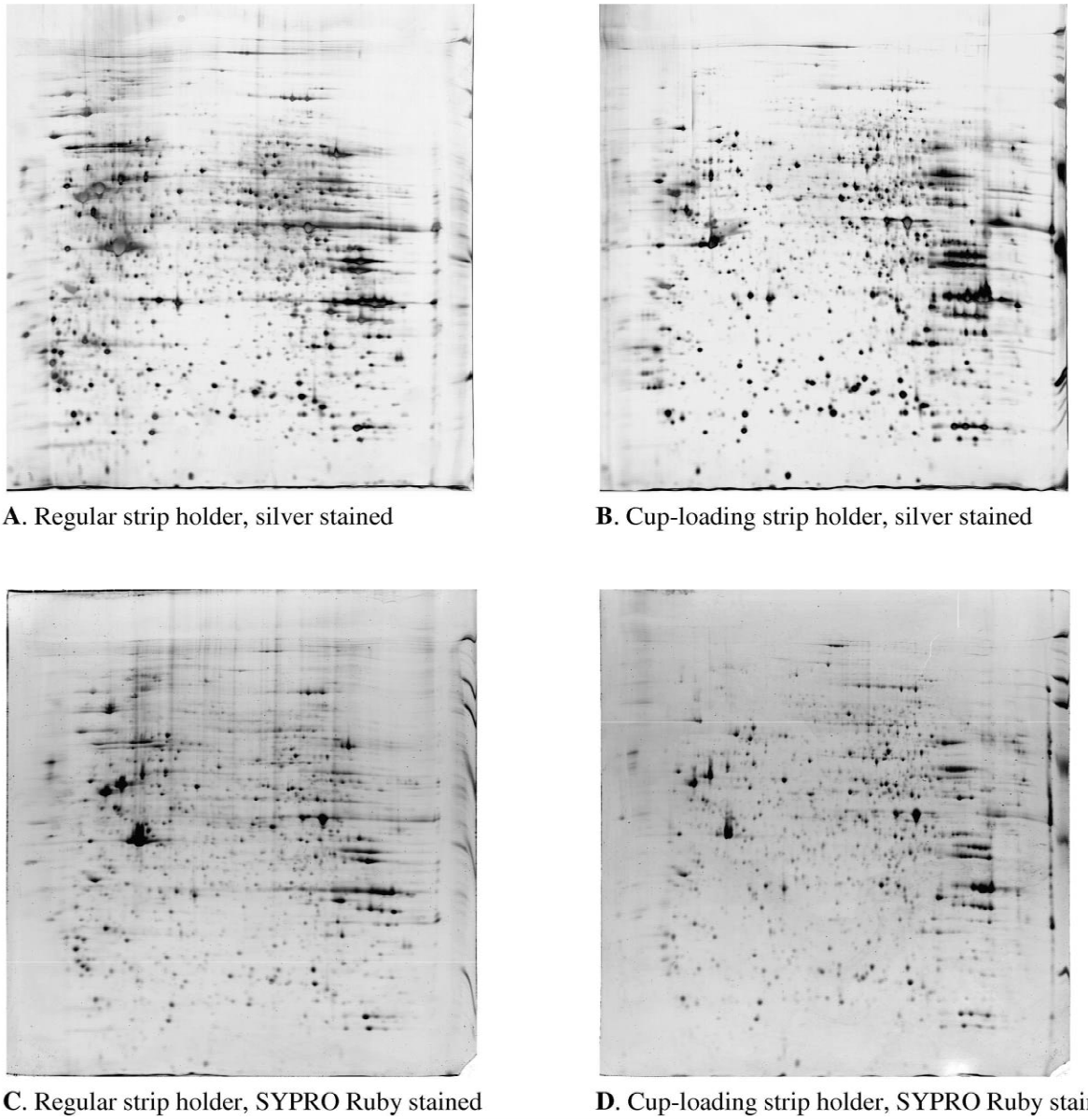

Fig. 2. Comparison of in-gel rehydration versus cup-loading, and silver versus fluorescence staining of HCC-M proteins. HCC-M cell lysate proteins were first separated in 18 cm Immobiline DryStrips pH 3–10 NL, using regular (A and C) and cup-loading strip holders (B and D). The proteins were then separated using 10%T SDS–PAGE gels in a PROTEAN II xi Cell IPG Conversion unit, at 30 mA/gel. The gels were silver stained (A and B) and SYPRO Ruby stained (C and D). Protein loading was ~120 μg/gel.

that sample application using the cup-loading method on the anode end of the IPG strips seemed to reduce the horizontal streaks to a certain extent (Fig. 2). This method was greatly facilitated by the recent release of the Universal strip holder for use on the IPGphor electrophoretic unit (Amersham Biosciences).

3.11. Fluorescent dyes versus silver staining

Silver staining is very sensitive (as low as 0.1 ng of protein per spot can be detected), but it is a multistep procedure with a very limited linear dynamic range. In addition it often leads to the formation of hollow spots or result in a doughnut effect which can complicate image analyses. On the other hand, staining with fluorescent dyes such as SYPRO Ruby is relatively sensitive, simple, and reproducible. In addition, it also has a broader linear dynamic range. We had compared the two staining methods for the 2-DE maps of HCC-M, and found SYPRO Ruby to be considerably less sensitive than silver staining (Fig. 2). Moreover, it was also found that some protein spots stained better with SYPRO Ruby than with silver nitrate, and the reverse was true as well. This observation is in full agreement with the report by Gorg et al. [18] who showed that the patterns obtained with silver staining and SYPRO Ruby staining were similar, but not identical. Finally, to facilitate the excision of the protein spots from 2-DE gels following SYPRO Ruby staining and image analysis, we have also developed a protocol to restain the gel with silver nitrate (results not shown).

3.12. Two-dimensional electrophoresis proteome database of HCC-M

3.12.1. Protein categorisation

We have earlier reported that, from a total of 408 unique spots excised from the 2-DE gel of HCC-M, 272 and 29 spots were identified by MALDI-TOF MS and nESI-MS–MS respectively [12,13]. This result represented the most comprehensive 2-DE protein database for any HCC cell line reported thus far. In this review, we have reorganised the database by grouping the proteins into different functional categories under (a) cell cycle, (b) chaperone/stress response, (c) cytoskeleton/mobility, (d) DNA replication/gene regulation, (e) immunological response, (f) ion channels, (g) membrane proteins, (h) metabolism, (i) oncogenes/tumour suppressor genes, (j) protection and detoxification, (k) protein synthesis and degradation, (l) signal transduction, (m) transport/binding proteins, (n) tumour associated proteins, and (o) unannotated/function inferred (Table 1). In addition, we have further grouped the proteins that have been shown to be implicated in HCC and other types of cancers into a separate list from the different categories of HCC-M proteins (see Table 2). We believe that such a categorisation and grouping of HCC-M proteins will simplify the 2DE protein database of HCC proteins, which in turn will facilitate the rapid identification and discovery of novel proteins that are involved in hepatocarcinogenesis.

3.12.2. HCC-M two-dimensional electrophoresis proteome web site

Finally, in line with our wish to allow the scientific community to access our extensive work on the identity of the proteins on the HCC-M 2-DE map, we have created an on-line database that provides an interactive way to query the HCC-M protein database. The data from our earlier publications [12,13] were first converted to MySQL database for data manipulation and retrieval. We are running an Apache web server and using Java servlets and applets technology to process and display the data.

There are two options to query the HCC-M database (Fig. 3).

Option 1: protein search by NCBI/SWISS-PROT Accession Number, Protein Name (full and partial name) and Protein ID (ID as published in our papers);

Option 2: interactive protein spots query on the original 2-DE image maps.

A query using Option 1 will retrieve a row or a list of proteins (if there are more than one match in the database) for selection as shown in Fig. 4. A click on the Sno column will display the protein identity page (Fig. 5) which includes information on the theoretical MW and p*I*, experimental MW and p*I*, a link to NCBI/SWISS-PROT information page, protein description, peptides matched from MALDI, subcellular location, method of identification and remarks. The location of the protein on the 2-DE map can also be

Table 1
Categorisation of identified proteins from the HCC-M cell line[a]

Accession no.[b]	Protein name(s)[c]
Cell cycle	
P34991	Cyclin A/CDK2-associated protein p19 (RNA polymerase II elongation factor-like protein) (organ of Corti protein 2) (OCP-II protein) (OCP-2) (transcription elongation factor B) (SIII)
Chaperone/stress response	
3273383	TRAP1
4758484	Glutathione-S-transferase like (glutathione transferase ω)
P04792	Heat shock 27 kDa protein (HSP 27) (stress-responsive protein 27) (SRP27) (oestrogen-regulated 24 kDa protein) (28 kDa heat shock protein)
P07900	Heat shock protein HSP 90-α (HSP 86)
P08107	Heat shock 70 kDa protein 1 (HSP70.1) (HSP70-1/HSP70-2)
P08238	Heat shock protein HSP 90-β (HSP 84) (HSP 90)
P10809	60 kDa Heat shock protein, mitochondrial precursor (Hsp60) (60 kDa chaperonin) (CPN60) (heat shock protein 60) (HSP-60) (mitochondrial matrix protein P1) (P60 lymphocyte protein) (HuCHA60)
P11021	78 kDa glucose-regulated protein precursor (GRP 78) (immunoglobulin heavy chain binding protein) (BIP) (endoplasmic reticulum lumenal Ca^{2+} binding protein grp78)
P11142	Heat shock cognate 71 kDa protein
P14625	Endoplasmin precursor (94 kDa glucose-regulated protein) (GRP94) (GP96 homolog) (tumour rejection antigen 1)
P30101	Protein disulphide isomerase A3 precursor (EC 5.3.4.1) (disulphide isomerase ER-60) (ERp60) (58 kDa microsomal protein) (P58) (ERp57)
P31948	Stress-induced-phosphoprotein 1 (STI1) (Hsp70/Hsp90-organizing protein) (transformation-sensitive protein IEF SSP 3521)
Cytoskeleton/mobility	
4502561	Capping protein (actin filament), gelsolin-like
P02545	Lamin A/C (70 kDa lamin)
P02570	Actin, cytoplasmic 1 (β-actin)
P02571	Actin, cytoplasmic 2 (γ-actin)
P04264	Keratin, type II cytoskeletal 1 (cytokeratin 1) (K1) (CK 1) (67 kDa cytokeratin) (hair α protein)
P07226	Tropomyosin, fibroblast non-muscle type (tropomyosin 4) (TM30-PL)
P08670	Vimentin
P08729	Keratin, type II cytoskeletal 7 (cytokeratin 7) (K7) (CK 7)
P09494	Tropomyosin α chain, fibroblast isoform TM3 (tropomyosin 1, fibroblast isoform TM3)
P12324	Tropomyosin, cytoskeletal type (tropomyosin 3, cytoskeletal) (TM30-NM)
P13797	T-Plastin
P32391	Actin-like protein 3 (actin-related protein 3) (actin-2)
P37802	Transgelin 2 (SM22-α homolog)
P40121	Macrophage capping protein (actin-regulatory protein CAP-G)
P42024	α-Centractin (centractin) (centrosome-associated actin homolog) (actin-RPV) (ARP1)
P47755	F-actin capping protein α-2 subunit (CAPZ α-2)
P47756	F-actin capping protein β subunit (CAPZ β)
P52565	Rho GDP-dissociation inhibitor 1 (rho GDI 1) (rho-GDI α)
P52907	F-actin capping protein α-1 subunit (CAPZ α-1)

Table 1. Continued

Accession no.[b]	Protein name(s)[c]
Q07960	Rho-GTPase-activating protein 1 (GTPase-activating protein rhoOGAP) (rho-related small GTPase protein activator) (CDC42 GTPase-activating protein) (P50-rhoGAP)
Q16658	Fascin (singed-like protein) (55 kDa actin bundling protein) (p55)
DNA replication/gene regulation	
542991	Ran-specific GTPase-activating protein
4504865	KH-type splicing regulatory protein (FUSE binding protein 2)
P09429	High mobility group protein HMG1 (HMG-1)
P12004	Proliferating cell nuclear antigen (PCNA) (cyclin)
P13010	ATP-dependent DNA helicase II, 80 kDa subunit (lupus Ku autoantigen protein p86) (Ku86) (Ku80) (86 kDa subunit of Ku antigen) (thyroid-lupus autoantigen) (TLAA) (CTC box binding factor 85 kDa subunit) (CTCBF) (CTC85) (nuclear factor IV) (DNA-repair protein XRCC5)
P15927	Replication protein A 32 kDa subunit (RP-A) (RF-A) (replication factor-A protein 2)
P35232	Prohibitin
P35250	Activator 1 40 kDa subunit (replication factor C 40 kDa subunit) (A1 40 kDa subunit) (RF-C 40 kDa subunit) (RFC40)
Q09028	Chromatin assembly factor 1 subunit C (CAF-1 subunit C) (chromatin assembly factor I p48 subunit) (CAF-I 48 kDa subunit) (CAF-Ip48) (retinoblastoma binding protein p48) (retinoblastoma-binding protein 4) (RBBP-4) (MSI1 protein homolog)
Q16576	Histone acetyltransferase type B subunit 2 (retinoblastoma binding protein P46) (retinoblastoma-binding protein 7) (RBBP-7)
Immunological response	
P09960	Leukotriene A-4 hydrolase (EC 3.3.2.6) (LTA-4 hydrolase) (leukotriene A(4) hydrolase)
P12815	Programmed cell death protein 6 (probable calcium-binding protein ALG-2) (PMP41) (ALG-257)
P17693	HLA class I histocompatibility antigen, α chain G precursor (HLA G antigen)
P30740	Leukocyte elastase inhibitor (LEI) (monocyte/neutrophil elastase inhibitor) (M/NEI) (EI)
Ion channels	
O00299	Chloride intracellular channel protein 1 (nuclear chloride ion channel 27) (NCC27) (P64 CLCP) (chloride channel ABP)
P21796	Voltage-dependent anion-selective channel protein 1 (VDAC-1) (hVDAC1) (outer mitochondrial membrane protein porin 1) (plasmalemmal porin) (porin 31HL) (porin 31HM)
P45880	Voltage-dependent anion-selective channel protein 2 (VDAC-2) (hVDAC2) (outer mitochondrial membrane protein porin 2)
Membrane proteins	
5174723	Mitochondrial outer membrane protein TOM40 (mitochondrial outer membrane protein)
Metabolism—amino acids	
2674062	3-Phosphoglycerate dehydrogenase
P00367	Glutamate dehydrogenase 1, mitochondrial precursor (EC 1.4.1.3) (GDH)
P00966	Argininosuccinate synthase (EC 6.3.4.5) (citrulline-aspartate ligase)
P12277	Creatine kinase, B chain (EC 2.7.3.2) (B-CK)
P19623	Spermidine synthase (EC 2.5.1.16) (putrescine aminopropyltransferase) (SPDSY)
P23526	Adenosylhomocysteinase (EC 3.3.1.1) (S-adenosyl-L-homocysteine hydrolase) (AdoHcyase)
P32322	Pyrroline-5-carboxylate reductase (EC 1.5.1.2) (P5CR) (P5C reductase)
P41250	Glycyl-tRNA synthetase (EC 6.1.1.14) (glycine–tRNA ligase) (GlyRS)[d]

Table 1. Continued

Accession no.[b]	Protein name(s)[c]
P48507	Glutamate-cysteine ligase regulatory subunit (EC 6.3.2.2) (γ-glutamylcysteine synthetase) (γ-ECS) (GCS light chain) (glutamate-cysteine ligase modifier subunit)
P48637	Glutathione synthetase (EC 6.3.2.3) (glutathione synthase) (GSH synthetase) (GSH-S)
P49419	Antiquitin (EC 1.2.1.-)
P49903	Selenide, water dikinase 1 (EC 2.7.9.3) (selenophosphate synthetase 1) (selenium donor protein 1)
Q13126	5′-Methylthioadenosine phosphorylase (EC 2.4.2.28) (MTA phosphorylase) (MTAPASE)
Metabolism—carbohydrate	
5174471	Isocitrate dehydrogenase 1 ($NADP^+$), soluble[d]
6694937	Nudix hydrolase NUDT5
9507063	*N*-acetylneuraminic acid phosphate synthase, sialic acid synthase
P00338	L-Lactate dehydrogenase A chain (EC 1.1.1.27) (LDH-A) (LDH muscle subunit) (LDH-M)[d]
P00558	Phosphoglycerate kinase 1 (EC 2.7.2.3) (primer recognition protein 2) (PRP 2)[d]
P00938	Triosephosphate isomerase (EC 5.3.1.1) (TIM)
P04075	Fructose-bisphosphate aldolase A (EC 4.1.2.13) (muscle-type aldolase) (lung cancer antigen NY-LU-1)[d]
P04406	Glyceraldehyde 3-phosphate dehydrogenase, liver (EC 1.2.1.12)[d]
P06733	α-Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydrolyase) (NON-neural enolase) (NNE) (Phosphopyruvate hydratase)[d]
P07195	L-Lactate dehydrogenase B chain (EC 1.1.1.27) (LDH-B) (LDH heart subunit) (LDH-H)[d]
P07954	Fumarate hydratase, mitochondrial precursor (EC 4.2.1.2) (fumarase)[d]
P09329	Ribose-phosphate pyrophosphokinase I (EC 2.7.6.1) (phosphoribosyl pyrophosphate synthetase I) (PPRibP) (PRS-I)[d]
P11413	Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49) (G6PD)
P11908	Ribose-phosphate pyrophosphokinase II (EC 2.7.6.1) (phosphoribosyl pyrophosphate synthetase II) (PPRibP) (PRS-II)[d]
P13929	β-Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydrolyase) (skeletal muscle enolase) (MSE)[d]
P14550	Alcohol dehydrogenase [$NADP^+$] (EC 1.1.1.2) (aldehyde reductase)[d]
P14618	Pyruvate kinase, M1 isozyme (EC 2.7.1.40) (pyruvate kinase muscle isozyme) (cytosolic thyroid hormone-binding protein) (CTHBP) (THBP1)[d]
P14786	Pyruvate kinase, M2 isozyme (EC 2.7.1.40)[d]
P18669	Phosphoglycerate mutase, brain form (EC 5.4.2.1) (PGAM-B) (EC 5.4.2.4) (EC 3.1.3.13) (BPG-dependent PGAM)
P29401	Transketolase (EC 2.2.1.1) (TK)[d]
P37837	Transaldolase (EC 2.2.1.2)
P40925	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37)[d]
P50213	Isocitrate dehydrogenase [NAD] subunit α, mitochondrial precursor (EC 1.1.1.41) (isocitric dehydrogenase) (NAD^+-specific ICDH)
P51570	Galactokinase (EC 2.7.1.6) (galactose kinase)
Q04760	Lactoylglutathione lyase (EC 4.4.1.5) (methylglyoxalase) (aldoketomutase) (glyoxalase I) (Glx I) (ketone-aldehyde mutase) (S-D-lactoylglutathione methylglyoxal lyase)
Q99798	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (citrate hydrolyase) (aconitase)[d]
Metabolism—cofactors and vitamins	
O00764	Pyridoxine kinase (EC 2.7.1.35) (pyridoxal kinase)

Table 1. Continued

Accession no.[b]	Protein name(s)[c]
P30043	Flavin reductase (EC 1.6.99.1) (FR) (NADPH-dependent diaphorase) (NADPH-flavin reductase) (FLR) (biliverdin reductase B) (EC 1.3.1.24) (BVR-B) (biliverdin-IX β-reductase) (green haem binding protein) (GHBP)
Metabolism–energy	
P06576	ATP synthase β chain, mitochondrial precursor (EC 3.6.3.14)
P13804	Electron transfer flavoprotein α-subunit, mitochondrial precursor (α-ETF)
P22695	Ubiquinol-cytochrome C reductase complex core protein 2, mitochondrial precursor (EC 1.10.2.2) (complex III subunit II)
Q15181	Inorganic pyrophosphatase (EC 3.6.1.1) (pyrophosphate phospho-hydrolase) (PPase)
Metabolism—lipid	
5174389	Acetyl-coenzyme A acetyltransferase 2 (acetoacetyl coenzyme A thiolase) (acetoacetyl coenzyme A thiolase)[d]
O00154	Cytosolic acyl coenzyme A thioester hydrolase (EC 3.1.2.2) (long chain acyl-CoA thioester hydrolase) (CTE-II) (brain acyl-CoA hydrolase) (BACH)
P02647	Apolipoprotein A-I precursor (Apo-AI)
P42126	3,2-*trans*-enoyl-CoA isomerase, mitochondrial precursor (EC 5.3.3.8) (dodecenoyl-CoA δ-isomerase)
P54619	5′-AMP-activated protein kinase, γ-1 subunit (AMPK γ-1 chain) (AMPKg)
P55809	Succinyl-CoA:3-ketoacid-coenzyme A transferase, mitochondrial precursor (EC 2.8.3.5) (succinyl CoA:3-oxoacid CoA-transferase)[d]
Q99714	3-Hydroxyacyl-CoA dehydrogenase type II (EC 1.1.1.35) (Type II HADH) (endoplasmic reticulum-associated amyloid β-peptide binding protein) (short-chain type dehydrogenase/reductase XH98G2)[d]
Metabolism—nucleotide	
P00491	Purine nucleoside phosphorylase (EC 2.4.2.1) (inosine phosphorylase) (PNP)[d]
P00568	Adenylate kinase isoenzyme 1 (EC 2.7.4.3) (ATP–AMP transphosphorylase) (AK1) (myokinase)
P07741	Adenine phosphoribosyltransferase (EC 2.4.2.7) (APRT)
P12268	Inosine-5′-monophosphate dehydrogenase 2 (EC 1.1.1.205) (IMP dehydrogenase 2) (IMPDH-II) (IMPD 2)
P15531	Nucleoside diphosphate kinase A (EC 2.7.4.6) (NDK A) (NDP kinase A) (tumour metastatic process-associated protein) (metastasis inhibition factor nm23) (nm23-H1)
P49915	GMP synthase [glutamine-hydrolyzing] (EC 6.3.5.2) (glutamine amidotransferase) (GMP synthetase)[d]
P55263	Adenosine kinase (EC 2.7.1.20) (AK) (adenosine 5′-phosphotransferase)
Oncogenes/tumour suppressor genes	
4503801	Far upstream element-binding protein (far upstream element binding protein) (FUSE-binding protein)
6005749	RNA-binding protein regulatory subunit
9910460	Nit protein 2
P36952	Maspin precursor (protease inhibitor 5)
Protection and detoxification	
2135069	Probable thioredoxin peroxidase (EC 1.11.1.-)
4507149	Superoxide dismutase 1, soluble [amyotrophic lateral sclerosis 1 (adult)] (Cu/Zn superoxide dismutase)
P00441	Superoxide dismutase [Cu–Zn]
P04179	Superoxide dismutase [Mn], mitochondrial precursor (EC 1.15.1.1)

Table 1. Continued

Accession no.[b]	Protein name(s)[c]
P08758	Annexin V (lipocortin V) (endonexin II) (calphobindin I) (CBP-I) (placental anticoagulant protein I) (PAP-I) (PP4) (thromboplastin inhibitor) (vascular anticoagulant-α) (VAC-α) (anchorin CII)
P09211	Glutathione S-transferase P (EC 2.5.1.18) (GST class-PI) (GSTP1-1)
P30041	Antioxidant protein 2 (1-Cys peroxiredoxin) (1-Cys PRX) (acidic calcium-independent phospholipase A2) (EC 3.1.1.-) (aiPLA2) (non-selenium glutathione peroxidase) (EC 1.11.1.7) (NSGPx) (24 kDa protein) (liver 2D PAGE spot 40) (red blood cells PAGE spot 12)
P30048	Thioredoxin-dependent peroxide reductase, mitochondrial precursor (peroxiredoxin 3) (antioxidant protein 1) (AOP-1) (MER5 protein homolog) (HBC189) (PRX III)
P32119	Peroxiredoxin 2 (thioredoxin peroxidase 1) (thioredoxin-dependent peroxide reductase 1) (thiol-specific antioxidant protein) (TSA) (PRP) (natural killer cell enhancing factor B) (NKEF-B)
P38646	Stress-70 protein, mitochondrial precursor (75 kDa glucose regulated protein) (GRP 75) (peptide-binding protein 74) (PBP74) (mortalin) (MOT)
Q06830	Peroxiredoxin 1 (thioredoxin peroxidase 2) (thioredoxin-dependent peroxide reductase 2) (proliferation-associated protein PAG) (natural killer cell enhancing factor A) (NKEF-A)
Protein synthesis and degradation	
542852	hnRNP protein E1
3986482	Translation initiation factor eIF3 p40 subunit (eIF3p40)
4468218	unr-interacting protein
4503519	Eukaryotic translation initiation factor 3, subunit 5 (ϵ, 47 kDa)
4506195	Proteasome (prosome, macropain) subunit, β type, 2 (proteasome subunit, β type, 2)
4506217	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 10
4506223	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 13 (hypothetical protein) (26S proteasome subunit p40.5)
4506237	Proteasome activator HPA28 subunit β
4506753	TATA binding protein interacting protein 49 kDa
5031981	26S proteasome-associated pad1 homolog
5031997	Proteasome (prosome, macropain) activator subunit 3 (PA28 γ, Ki) (Ki nuclear autoantigen)
5174731	Translin-associated factor X
P04632	Calcium-dependent protease, small subunit (calpain regulatory subunit) (calcium-activated neutral proteinase) (CANP)
P04720	Elongation factor 1-α 1 (EF-1-α-1) (elongation factor 1 A-1) (eEF1A-1) (elongation factor Tu) (EF-Tu)
P05198	Eukaryotic translation initiation factor 2 subunit 1 (eukaryotic translation initiation factor 2 α subunit) (eIF-2-α) (EIF-2α) (EIF-2A)
P07237	Protein disulphide isomerase precursor (PDI) (EC 5.3.4.1) (prolyl 4-hydroxylase β subunit) (cellular thyroid hormone binding protein) (P55)
P07602	Proactivator polypeptide precursor [Contains: saposin A (protein A); saposin B (sphingolipid activator protein 1) (SAP-1) (cerebroside sulphate activator) (CSAct) (dispersin) (sulphatide/GM1 activator); saposin C (Co-β-glucosidase) (A1 activator) (glucosylceramidase activator) (sphingolipid activator protein 2) (SAP-2); saposin D (protein C) (component C)]
P08865	40S ribosomal protein SA (P40) (34/67 kDa laminin receptor) (colon carcinoma laminin-binding protein) (NEM/1CHD4)

Table 1. Continued

Accession no.[b]	Protein name(s)[c]
P09651	Heterogeneous nuclear ribonucleoprotein A1 (helix-destabilizing protein) (single-strand binding protein) (hnRNP core protein A1)
P12081	Histidyl-tRNA synthetase (EC 6.1.1.21) (histidine–tRNA ligase) (HisRS)
P13639	Elongation factor 2 (EF-2)
P13798	Acylamino-acid-releasing enzyme (EC 3.4.19.1) (acyl-peptide hydrolase) (APH) (acylaminoacyl-peptidase) (DNF15S2 protein)
P14866	Heterogeneous nuclear ribonucleoprotein L (hnRNP L)
P15374	Ubiquitin carboxyl-terminal hydrolase isozyme L3 (EC 3.4.19.12) (UCH-L3) (ubiquitin thiolesterase L3)
P17987	T-complex protein 1, α subunit (TCP-1-α) (CCT-α)
P22061	Protein-L-isoaspartate(D-aspartate) O-methyltransferase (EC 2.1.1.77) (protein-β-aspartate methyltransferase) (PIMT) (protein L-isoaspartyl/D-aspartyl methyltransferase) (L-isoaspartyl protein carboxyl methyltransferase)
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/hnRNP B1)
P25786	Proteasome subunit α type 1 (EC 3.4.25.1) (proteasome component C2) (macropain subunit C2) (multicatalytic endopeptidase complex subunit C2) (proteasome ν chain) (30 kDa prosomal protein) (PROS-30)
P25787	Proteasome subunit alpha type 2 (EC 3.4.25.1) (proteasome component C3) (macropain subunit C3) (multicatalytic endopeptidase complex subunit C3)
P25789	Proteasome subunit alpha type 4 (EC 3.4.25.1)(proteasome component C9) (macropain subunit C9) (multicatalytic endopeptidase complex subunit C9) (proteasome subunit L)
P27924	Ubiquitin-conjugating enzyme E2-25 kDa (EC 6.3.2.19) (ubiquitin–protein ligase) (ubiquitin carrier protein) (Huntington interacting protein) (HIP-2)
P28070	proteasome subunit β type 4 precursor (EC 3.4.25.1) (proteasome β-chain) (macropain β chain) (multicatalytic endopeptidase complex β-chain) (proteasome chain 3) (HSN3) (HsBPROS26)
P28072	Proteasome subunit β type 6 precursor (EC 3.4.25.1) (proteasome δ-chain) (macropain δ chain) (multicatalytic endopeptidase complex δ chain) (proteasome subunit Y)
P30040	Endoplasmic reticulum protein ERp29 precursor (ERp31) (ERp28)
P31943	Heterogeneous nuclear ribonucleoprotein H (hnRNP H)
P33240	Cleavage stimulation factor, 64 kDa subunit (CSTF 64 kDa subunit) (CF-1 64 kDa subunit)
P34062	Proteasome subunit α type 6 (EC 3.4.25.1) (proteasome ι chain) (macropain ι chain) (multicatalytic endopeptidase complex ι chain) (27 kDa prosomal protein) (PROS-27) (p27K)
P35237	Placental thrombin inhibitor (cytoplasmic antiproteinase) (CAP) (protease inhibitor 6)
P35998	26S protease regulatory subunit 7 (MSS1 protein)
P40227	T-complex protein 1, ζ subunit (TCP-1-ζ) (CCT-ζ) (CCT-ζ-1) (Tcp20) (HTR3)
P48643	T-complex protein 1, ϵ subunit (TCP-1-ϵ) (CCT-ϵ)
P49368	T-complex protein 1, γ subunit (TCP-1-γ) (CCT-γ)
P49411	Elongation factor Tu, mitochondrial precursor (P43)
P49720	Proteasome subunit β type 3 (EC 3.4.25.1) (proteasome θ chain) (proteasome chain 13) (proteasome component C10-II)
P50990	T-complex protein 1, θ subunit (TCP-1-θ) (CCT-θ)
P50991	T-complex protein 1, δ subunit (TCP-1-δ) (CCT-δ) (stimulator of TAR RNA binding)
P55795	Heterogeneous nuclear ribonucleoprotein H′ (hnRNP H′) (FTP-3)

Table 1. Continued

Accession no.[b]	Protein name(s)[c]
Q06323	Proteasome activator complex subunit 1 (proteasome activator 28-α subunit) (PA28α) (PA28a) (activator of multicatalytic protease subunit 1) (11S regulator complex α subunit) (REG-α) (interferon γ upregulated I-5111 protein) (IGUP I-5111)
Q07244	Heterogeneous nuclear ribonucleoprotein K (hnRNP K) (DC-stretch binding protein) (CSBP) (transformation upregulated nuclear protein) (TUNP)
Q13347	Eukaryotic translation initiation factor 3 subunit 2 (eIF-3 β) (eIF3 p36) (TGF-β receptor interacting protein 1) (TRIP-1)
Q16740	Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial precursor (EC 3.4.21.92) (endopeptidase Clp)
Q99832	T-complex protein 1, η subunit (TCP-1-η) (CCT-η) (HIV-1 Nef interacting protein)
Signal transduction	
1082693	Phosphotyrosyl phosphatase activator PTPA
1244400	hCRMP-2
3309170	COP9 complex subunit 4
4757834	BCL2-associated athanogene 2 (BAG-family molecular chaperone regulator-2)
4827056	WD repeat-containing protein 1, isoform 2
P04083	Annexin I (lipocortin I) (calpactin II) (chromobindin 9) (P35) (phospholipase A2 inhibitory protein)
P04901	Guanine nucleotide-binding protein G(I)/G(S)/G(T) β subunit 1 (transducin β chain 1)
P07355	Annexin II (lipocortin II)(calpactin I heavy chain) (chromobindin 8)(P36)(protein I)(placental anticoagulant protein IV)(PAP-IV)
P11016	Guanine nucleotide-binding protein G(I)/G(S)/G(T) β subunit 2 (transducin β chain 2) (G protein β 2 subunit)
P25388	Guanine nucleotide-binding protein β subunit-like protein 12.3 (P205) (receptor of activated protein kinase C 1) (RACK1) (receptor for activated C kinase) (GNB2-RS1)
P29312	14-3-3 Protein ζ/δ (protein kinase C inhibitor protein-1) (KCIP-1) (factor activating exoenzyme S) (FAS)
P29354	Growth factor receptor-bound protein 2 (GRB2 adapter protein) (SH2/SH3 adapter GRB2) (ASH protein)
P30086	Phosphatidylethanolamine-binding protein (PEBP) (neuropolypeptide h3) (hippocampal cholinergic neurostimulating peptide) (HCNP) (raf kinase inhibitor protein) (RKIP)
P30153	Serine/threonine protein phosphatase PP2A, 65 kDa regulatory unit, α-isoform (PP2A, subunit A, PR65-α isoform) (PP2A, subunit A, R1-α isoform) (medium tumour antigen-associated 61 kDa protein)
P42655	14-3-3 Protein ε (mitochondrial import stimulation factor L subunit) (protein kinase C inhibitor protein-1) (KCIP-1) (14-3-3E)
P51692	Signal transducer and activator of transcription 5B
Q00688	Rapamycin-selective 25 kDa immunophilin (FKBP25) (peptidyl-prolyl *cis–trans* isomerase) (EC 5.2.1.8) (PPiase) (rotamase)
Transport/binding proteins	
4503013	Copine I
P02787	Serotransferrin precursor (siderophilin) (β-1-metal binding globulin)
P17080	GTP-binding nuclear protein RAN (TC4) (RAN GTPase) (androgen receptor-associated protein 24)

Table 1. Continued

Accession no.[b]	Protein name(s)[c]
P17931	Galectin-3 (galactose-specific lectin 3) (MAC-2 antigen) (IgE-binding protein) (35 kDa lectin) (carbohydrate binding protein 35) (CBP 35) (laminin-binding protein) (lectin L-29) (L-31) (galactoside-binding protein) (GALBP)
P54920	α-Soluble NSF attachment protein (SNAP-α) (*N*-ethylmaleimide-sensitive factor attachment protein, α)
Q02790	FK506-binding protein 4 (possible peptidyl-prolyl *cis–trans* isomerase FKBP4) (EC 5.2.1.8) (PPiase) (rotamase) (p59 protein) (HSP binding immunophilin) (HBI) (FKBP52 protein) (52 kDa FK506 binding protein) (FKBP59)
Tumour associated proteins	
P13693	Translationally controlled tumour protein (TCTP) (p23) (histamine-releasing factor) (HRF)
Unannotated/function inferred	
509033	GARS protein
2984585	P1.11659_4
2135068	Enhancer protein
3420179	WDR1 protein
3646128	Thioredoxin-like protein
3882167	KIAA0723 protein
4468253	A6 related protein
9966764	Lysophospholipase II
P12429	Annexin III (lipocortin III) (placental anticoagulant protein III) (PAP-III) (35-α calcimedin) (inositol 1,2-cyclic phosphate 2-phosphohydrolase)
Q61990	Poly(rC)-binding protein 2 (α-CP2) (putative heterogeneous nuclear ribonucleoprotein X) (hnRNP X) (CTBP) (CBP)

[a] Based on the list of proteins identified in our earlier papers [12,13]. Note that protein names are according to the latest updates in the NCBI and SWISS-PROT databases, which may differ from the names reported in the previous papers, but the accession numbers remain the same.
[b] The proteins are sorted according to accession numbers within each category.
[c] Names in brackets are synonyms.
[d] Proteins that are involved in more than one metabolic pathway.

Table 2
List of HCC-M proteins implicated in HCC and other cancers

Protein name(s)	Accession no.	References
Chaperone/stress induced		
Heat shock 27 kDa protein (HSP 27) (stress-responsive protein 27) (SRP27) (oestrogen-regulated 24 kDa protein) (28 kDa heat shock protein)	P04792	L.Yu et al., Electrophoresis, 21 (2000) 3058. Identification of differentially expressed proteins between human hepatoma and normal liver cell lines by two-dimensional electrophoresis and liquid chromatography–ion trap mass spectrometry
Heat shock protein HSP 90-α HSP 86)	P07900	J. Hu and C. Seeger, Proc Natl Acad Sci USA 93 (1996) 1060. Hsp90 is required for the activity of a hepatitis B virus reverse transcriptase G. Cho et al., Biochem Biophys Res Commun, 269 (2000) 191. Localization of HSP90 binding in the human hepatitis B virus polymerase

Table 2. Continued

Protein name(s)	Accession no.	References
Heat shock 70 kDa protein 1 (HSP70.1) (HSP70-1/HSP70-2)	P08107	M. Hantschel et al., Cell Stress Chaperones, 5 (2000) 438. Hsp70 plasma membrane expression on primary tumor biopsy material and bone marrow of leukemic patients
60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa chaperonin) (CPN60) (heat shock protein 60) (HSP-60) (mitochondrial matrix protein P1) (P60 lymphocyte protein) (HuCHA60)	P10809	J. Schneider et al., Anticancer Res, 19 (1999) 2141. Immunohistochemical detection of HSP60-expression in human ovarian cancer. Correlation with survival in a series of 247 patients
Heat shock cognate 71 kDa protein	P11142	I. Byrjalsen et al., Mol Hum Reprod, 5 (1999) 748. Two-dimensional gel analysis of human endometrial proteins: characterization of proteins with increased expression in hyperplasia and adenocarcinoma
Endoplasmin precursor (94 kDa glucose-regulated protein) (GRP94) (GP96 homolog) (tumour rejection antigen 1)	P14625	A. Menoret et al., Int J Cancer, 56 (1994) 400. Expression of the 100 kDa glucose-regulated protein (GRP100/endoplasmin) is associated with tumorigenicity in a model of rat colon adenocarcinoma
Cytoskeleton/mobility		
Keratin, type II cytoskeletal 7 (Cytokeratin 7) (K7) (CK 7)	P08729	P. Van Eyken et al., Histopathology, 17 (1990) 101. Abundant expression of cytokeratin 7 in fibrolamellar carcinoma of the liver
Tropomyosin α chain, fibroblast isoform TM3 (tropomyosin 1, fibroblast isoform TM3)	P09494	P.J. Wirth, Electrophoresis. 15 (1994) 358. Two-dimensional polyacrylamide gel electrophoresis in experimental hepatocarcinogenesis studies
Fascin (singed-like protein) (55 kDa actin bundling protein) (p55)	Q16658	W. Hu et al., Clin Exp Metastasis, 18 (2000) 83. Increased expression of fascin, motility associated protein, in cell cultures derived from ovarian cancer and in borderline and carcinomatous ovarian tumors
DNA replication/gene regulation		
High mobility group protein HMG1 (HMG-1)	P09429	K. Kajino et al., Intervirology 44 (2001) 311. Recombination hot spot of hepatitis B virus genome binds to members of the HMG domain protein family and the Y box binding protein family; implication of these proteins in genomic instability N. Kawahara et al., Cancer Res, 56 (1996) 5330. Enhanced coexpression of thioredoxin and high mobility group protein 1 genes in human hepatocellular carcinoma and the possible association with decreased sensitivity to cisplatin
Proliferating cell nuclear antigen (PCNA) (cyclin)	P12004	L. Nakopoulou et al., Pathol Res Pract, 191 (1995) 1208. Immunohistochemical expression of p53 protein and proliferating cell nuclear antigen in hepatocellular carcinoma T. Suehiro et al., Cancer, 76 (1995) 399. Clinicopathologic features and prognosis of resected hepatocellular carcinomas of varied sizes with special reference to proliferating cell nuclear antigen
Prohibitin	P35232	S. Tanno et al., Jpn J Cancer Res, 88 (1997) 1155. Prohibitin expression is decreased in the regenerating liver but not in chemically induced hepatic tumors in rats T. Sato et al., Genomics, 17 (1993) 762. The human prohibitin (PHB) gene family and its somatic mutations in human tumors

Table 2. Continued

Protein name(s)	Accession no.	References
Immunological response		
HLA class I histocompatibility antigen, α chainG precursor (HLA G antigen)	P17693	D.H. Moore et al., Gynecol Oncol, 38 (1990) 458. Class I histocompatibility antigen expression: a prognostic factor for aneuploid ovarian cancers
Metabolism		
3-Phosphoglycerate dehydrogenase	2674062	K. Snell et al., Biochem J. 245 (1987) 609. The modulation of serine metabolism in hepatoma 3924a during different phases of cellular proliferation in culture K. Snell and G. Weber, Biochem J, 233 (1986) 617. Enzymic imbalance in serine metabolism in rat hepatomas
Isocitrate dehydrogenase 1 ($NADP^+$), soluble	5174471	A.N. Murphy et al., Biochem Biophys Res Commun, 157 (1988) 1218. Calcium sensitive isocitrate and 2-oxoglutarate dehydrogenase activities in rat liver and AS-30D hepatoma mitochondria
Purine nucleoside phosphorylase (EC 2.4.2.1) (inosine phosphorylase) (PNP)	P00491	T.U. Krohne et al., Hepatology, 34 (2001) 511. Mechanisms of cell death induced by suicide genes encoding purine nucleoside phosphorylase and thymidine kinase in human hepatocellular carcinoma cells in vitro L. Mohr et al., Hepatology, 31 (2000) 606. Gene therapy of hepatocellular carcinoma in vitro and in vivo in nude mice by adenoviral transfer of the *Escherichia coli* purine nucleoside phosphorylase gene O. Sanfilippo et al., Cancer Biochem Biophys, 14 (1994) 57. Relationship between the levels of purine salvage pathway enzymes and clinical/biological aggressiveness of of human colon carcinoma
Triosephosphate isomerase (EC 5.3.1.1) (TIM)	P00938	T. Nagase et al., Comp Biochem Physiol B, 99 (1991) 193. Analyses of polypeptides in the liver of a novel mutant (LEC rats) to hereditary hepatitis and hepatoma by two-dimensional gel electrophoresis: identification of P29/6.8 as carbonic anhydrase III and triosephosphate isomerase
Glyceraldehyde-3-phosphate dehydrogenase, liver (EC 1.2.1.12)	P04406	Y. Gong et al., Hepatology, 23 (1996) 734. Comparison of glyceraldehyde-3-phosphate dehydrogenase and 28s-ribosomal RNA gene expression in human hepatocellular carcinoma as carbonic anhydrase III and triosephosphate isomerase
ATP synthase β chain, mitochondrial precursor (EC 3.6.3.14)	P06576	F. Capuano et al., J Bioenerg Biomembr, 29 (1997) 379. Oxidative phosphorylation enzymes in normal and neoplastic cell growth
α-Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydrolyase) (NON-neural enolase) (NNE) (phosphopyruvate hydratase)	P06733	N. Durany et al., Br J Cancer, 75 (1997) 969. Phosphoglycerate mutase, 2,3-bisphosphoglycerate phosphatase and enolase activity and isoenzymes in lung, colon and liver carcinomas and neoplastic cell growth
Inosine-5′-monophosphate dehydrogenase 2 (EC 1.1.1.205) (IMP dehydrogenase 2) (IMPDH-II) (IMPD 2)	P12268	H.N. Jayaram et al., Curr Med Chem, 6 (1999) 561. Consequences of IMP dehydrogenase inhibition, and its relationship to cancer and apoptosis
Creatine kinase, B-chain (EC 2.7.3.2) (B-CK)	P12277	J. Joseph et al., Br J Cancer, 76 (1997) 600. Creatine kinase activity and isoenzymes in lung, colon and liver carcinomas

Table 2. Continued

Protein name(s)	Accession no.	References
5′-Methylthioadenosine phosphorylase (EC 2.4.2.28) (MTA phosphorylase) (MTAPASE)	Q13126	M. Schmid et al., Oncogene, 19 (2000) 5747. A methylthioadenosine phosphorylase (MTAP) fusion transcript identifies a new gene on chromosome 9p21 that is frequently deleted in cancer F. Della Ragione et al., Oncogene, 10 (1995) 827. 5′-Deoxy-5′-methylthioadenosine phosphorylase and p16INK4 deficiency in multiple tumor cell lines
Alcohol dehydrogenase [$NADP^+$] (EC 1.1.1.2) (aldehyde reductase)	P14550	Z. Zhang and J. Bian, Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 18 (2001) 62. [in Chinese] [Progress in researches on the relationship between genetic polymorphisms of alcohol-metabolizing enzymes and cancers]
Nucleoside diphosphate kinase A (EC 2.7.4.6) (NDK A) (NDP kinase A) (tumour metastatic process-associated protein) (metastasis inhibition factor nm23) (nm23-H1)	P15531	Y. Fujimoto et al., J Gastroenterol, 33 (1998) 368. Reduced expression and rare genomic alteration of nm23-H1 in human hepatocellular carcinoma and hepatoma cell lines N. Iizuka et al., Cancer Res, 55 (1995) 652. NM23-H1 and NM23-H2 messenger RNA abundance in human hepatocellular carcinoma
Phosphoglycerate mutase, brain form (EC 5.4.2.1) (PGAM-B) (EC 5.4.2.4) (EC 3.1.3.13) (BPG-dependent PGAM)	P18669	N. Durany et al., Br J Cancer, 75 (1997) 969. Phosphoglycerate mutase, 2,3-bisphosphoglycerate phosphatase and enolase activity and isoenzymes in lung, colon and liver carcinomas
Transaldolase (EC 2.2.1.2)	P37837	P.C. Heinrich et al., Cancer Res, 36 (1976) 3189. Behavior of transaldolase (EC 2.2.1.2) and transketolase (EC 2.2.1.1) Activities in normal, neoplastic, differentiating, and regenerating liver
Glutathione synthetase (EC 6.3.2.3) (glutathione synthase) (GSH synthetase) (GSH-S)	P48637	Z. Huang et al., FASEB J, 15 (2001) 19. Mechanism and significance of increased glutathione level in human hepatocellular carcinoma and liver regeneration
3-Hydroxyacyl-CoA dehydrogenase type II (EC 1.1.1.35) (Type II HADH) (endoplasmic reticulum-associated amyloid β-peptide binding protein) (short-chain type dehydrogenase/reductase XH98G2)	Q99714	K. Suto et al., J Cancer Res Clin Oncol, 125 (1999) 83. Decreased expression of the peroxisomal bifunctional enzyme and carbonyl reductase in human hepatocellular carcinomas
Oncogenes/tumour suppressor genes		
RNA-binding protein regulatory subunit	6005749	D. Nagakubo et al. Biochem. Biophys. Res. Commun. (1997) 509. DJ-1, a novel oncogene that transformes mouse NIH3T3 cells in cooporation with ras
Maspin precursor (protease inhibitor 5)	P36952	L. Yu et al., Electrophoresis, 21 (2000) 3058. Identification of differentially expressed proteins between human hepatoma and normal liver cell lines by two-dimensional electrophoresis and liquid chromatography–ion trap mass spectrometry N. Maass et al., J Pathol, 195 (2001) 321. Decline in the expression of the serine proteinase inhibitor maspin is associated with tumour progression in ductal carcinomas of the breast

Table 2. Continued

Protein name(s)	Accession no.	References
Protection and detoxification		
Superoxide dismutase 1, soluble [amyotrophic lateral sclerosis 1 (adult)] (Cu/Zn superoxide dismutase)	4507149	M. Marikovsky et al., Int J Cancer, 97 (2002) 34. Cu/Zn superoxide dismutase plays a role in angiogenesis
Superoxide dismutase precursor (MN), mitochondrial precursor (EC 1.15.1.1)	P04179	V. Hajnicka et al., Acta Virol, 44 (2000) 343. Comparison of manganese superoxide dismutase precursor induction ability in human hepatoma cells with or without hepatitis B virus DNA insertion
Annexin V (lipocortin V) (endonexin II) (calphobindin I) (CBP-I) (placental anticoagulant protein I) (PAP-I) (PP4) (thromboplastin inhibitor) (vascular anticoagulant-α) (VAC-α) (anchorin CII)	P08758	Z.J. Gong et al., Hepatology, 29 (1999) 576. Transfection of a rat hepatoma cell line with a construct expressing human liver annexin V confers susceptibility to hepatitis B virus infection
Glutathione S-transferase P (EC 2.5.1.18) (GST class-PI) (GSTP1-1)	P09211	J.C. Tchou et al., Int J Oncol, 16 (2000) 663. GSTP1 CpG island DNA hypermethylation in hepatocellular carcinomas T. Zhou et al., Cancer Res, 57 (1997) 2749. Glutathione S-transferase expression in hepatitis B virus-associated human hepatocellular carcinogenesis
Peroxiredoxin 2 (thioredoxin peroxidase 1) (thioredoxin-dependent peroxide reductase 1) (thiol-specific antioxidant protein) (TSA) (PRP) (natural killer cell enhancing factor B) (NKEF-B)	P32119	D.Y. Noh et al., Anticancer Res, 21 (2001) 2085. Overexpression of peroxiredoxin in human breast cancer T. Yanagawa et al., Cancer Lett, 145 (1999) 127. Peroxiredoxin I expression in human thyroid tumors L.H. Butterfield et al., Antioxid Redox Signal, 1 (1999) 385. From cytoprotection to tumor suppression: the multifactorial role of peroxiredoxins
Protein synthesis and degradation		
Translation initiation factor eIF3 p40 subunit (eIF3p40)	3986482	N.N. Nupponen et al., Am J Pathol, 154 (1999) 1777. Amplification and overexpression of p40 subunit of eukaryotic translation initiation factor 3 in breast and prostate cancer
Eukaryotic translation initiation factor 3, subunit 5 (ϵ, 47000)	4503519	L. Lin et al., J Cell Biochem, 80 (2001) 483. Molecular interaction between human tumor marker protein p150, the largest subunit of eIF3, and intermediate filament protein K7
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 10	4506217	H. Higashitsuji et al., Nat Med, 6 (2000) 96. Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas S.A. Shah et al., Surg Oncol, 10 (2001) 43. Ubiquitin proteasome pathway: implications and advances in cancer therapy
Placental thrombin inhibitor (cytoplasmic antiproteinase) (CAP) (protease inhibitor 6)	P35237	S.D. Mikolajczyk et al., Cancer Res, 59 (1999) 3927. Identification of a novel complex between human kallikrein 2 and protease inhibitor-6 in prostate cancer tissue

Table 2. Continued

Protein name(s)	Accession no.	References
Signal transduction		
Annexin I (lipocortin I) (calpactin II) (chromobindin 9) (P35) (phospholipase A2 inhibitory protein)	P04083	C. de Coupade et al., Hepatology, 31 (2000) 371. Annexin 1 expression and phosphorylation are upregulated during liver regeneration and transformation in antithrombin III SV40 T large antigen transgenic mice T. Masaki et al., Hepatology, 24 (1996) 72. Enhanced expression of the protein kinase substrate annexin in human hepatocellular carcinoma
Guanine nucleotide-binding protein β subunit-like protein 12.3 (P205) (receptor of activated protein kinase C 1) (RACK1) (receptor for activated C kinase) (GNB2-RS1)	P25388	D. Schechtman et al., Oncogene, 20 (2001) 6339. Adaptor proteins in protein kinase C-mediated signal transduction
14-3-3 Protein ζ/δ (protein kinase C inhibitor protein-1) (KCIP-1) (factor activating exoenzyme S) (FAS)	P29312	N. Iwata et al., Oncogene, 19 (2000) 5298. Frequent hypermethylation of CpG islands and loss of expression of the 14-3-3 sigma gene in human hepatocellular carcinoma
Serine/threonine protein phosphatase PP2A, 65 kDa regulatory unit, α-isoform (PP2A, subunit A, PR65-α isoform) (PP2A, subunit A, (PP2A, subunit A, PR65-α isoform) (PP2A, subunit A, R1-α isoform) (medium tumour antigen-associated 61 kDa protein)	P30153	C. Fukukawa et al., Cancer Lett, 161 (2000) 89. Up-regulation of I-2(PP2A)/SET gene expression in rat primary hepatomas and regenerating livers R. Ruediger et al., Oncogene, 20 (2001) 10 Disruption of protein phosphatase 2A subunit interaction in human cancers with mutations in the A alpha subunit gene
14-3-3 Protein ϵ (mitochondrial import stimulation factor L subunit) (protein kinase C inhibitor protein-1) (KCIP-1) (14-3-3E)	P42655	N. Iwata et al., Oncogene, 19 (2000) 5298. Frequent hypermethylation of CpG islands and loss of expression of the 14-3-3 sigma gene in human hepatocellular carcinoma
Transport/binding proteins		
Galectin-3 (galactose-specific lectin 3) (MAC-2 antigen) (IgE-binding protein) (35 kDa lectin) (carbohydrate binding protein 35) (CBP 35) (laminin-binding protein) (lectin L-29) (L-31) (galactoside-binding protein) (GALBP)	P17931	T. Yoshii et al., J Biol Chem, 2001 (in press) Galectin-3 phosphorylation is required for its anti-apoptotic function and cell cycle arrest D.K. Hsu et al., Int J Cancer, 81 (1999) 519. Galectin-3 expression is induced in cirrhotic liver and hepatocellular carcinoma
Tumour associated proteins		
Translationally controlled tumour protein (TCTP) (p23) (histamine-releasing factor) (HRF)	P13693	J.C. Sanchez et al., Electrophoresis, 18 (1997) 150. Translationally controlled tumor protein: a protein identified in several non-tumoral cells including erythrocytes S. Chung et al., Cancer Lett, 156 (2000) 185. Expression of translationally controlled tumor protein mRNA in human colon cancer

retrieved by using the View Protein Spot Location button. Currently only one protein can be selected at any one time.

For Option 2, two image maps were used in the interactive protein spots query format: (i) a preparative 2-DE map (HCCM074) with a protein load of ~300 μg and (ii) an analytical 2-DE map (HCCM105) with a protein load of ~120 μg. Fig. 6 shows the 2-DE map of HCCM074 with the identified proteins labelled as red spots. Moving the mouse pointer over the spot will display its accession number, and the protein information (Fig. 5) can be

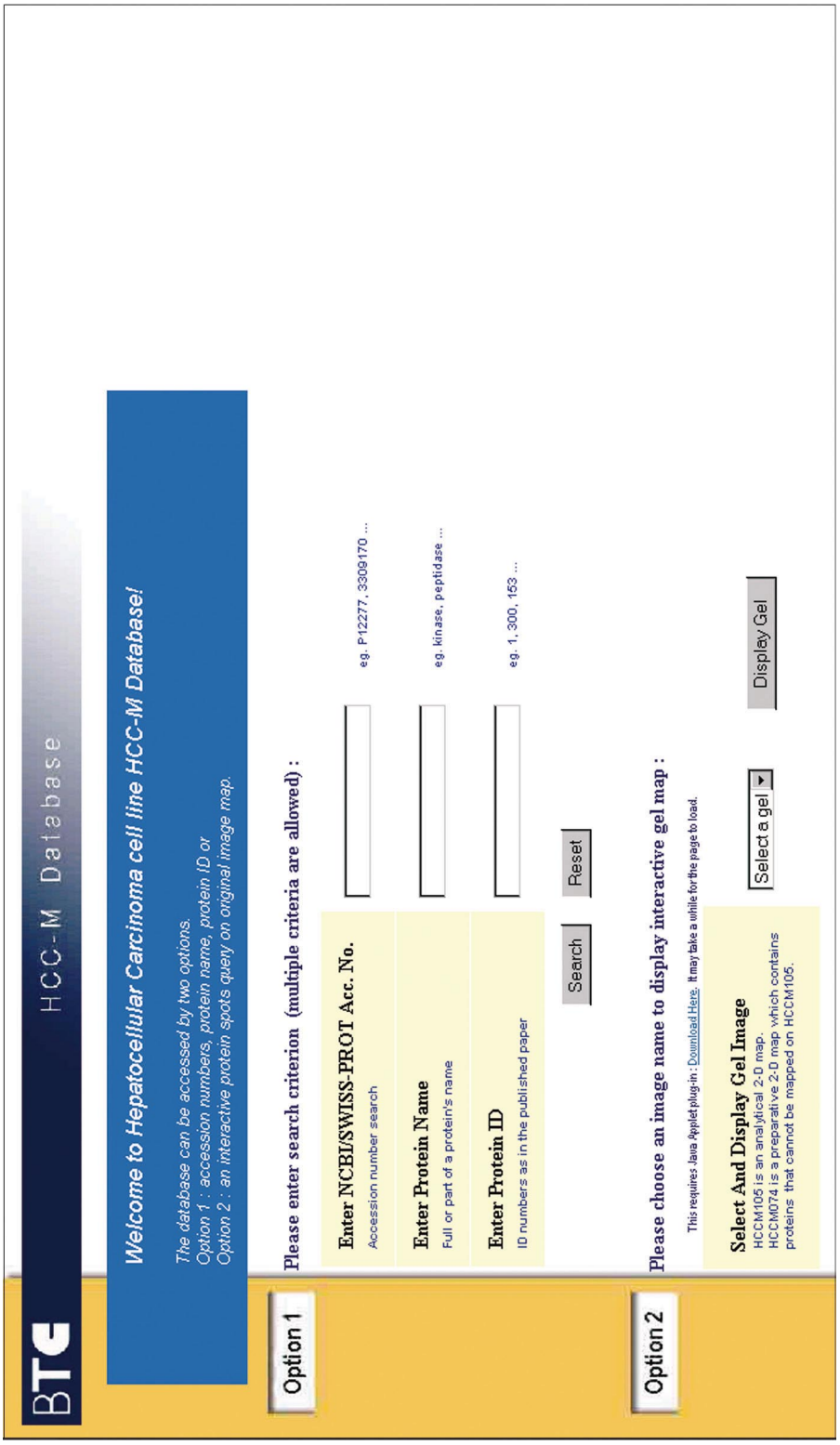

Fig. 3. HCC-M database main page.

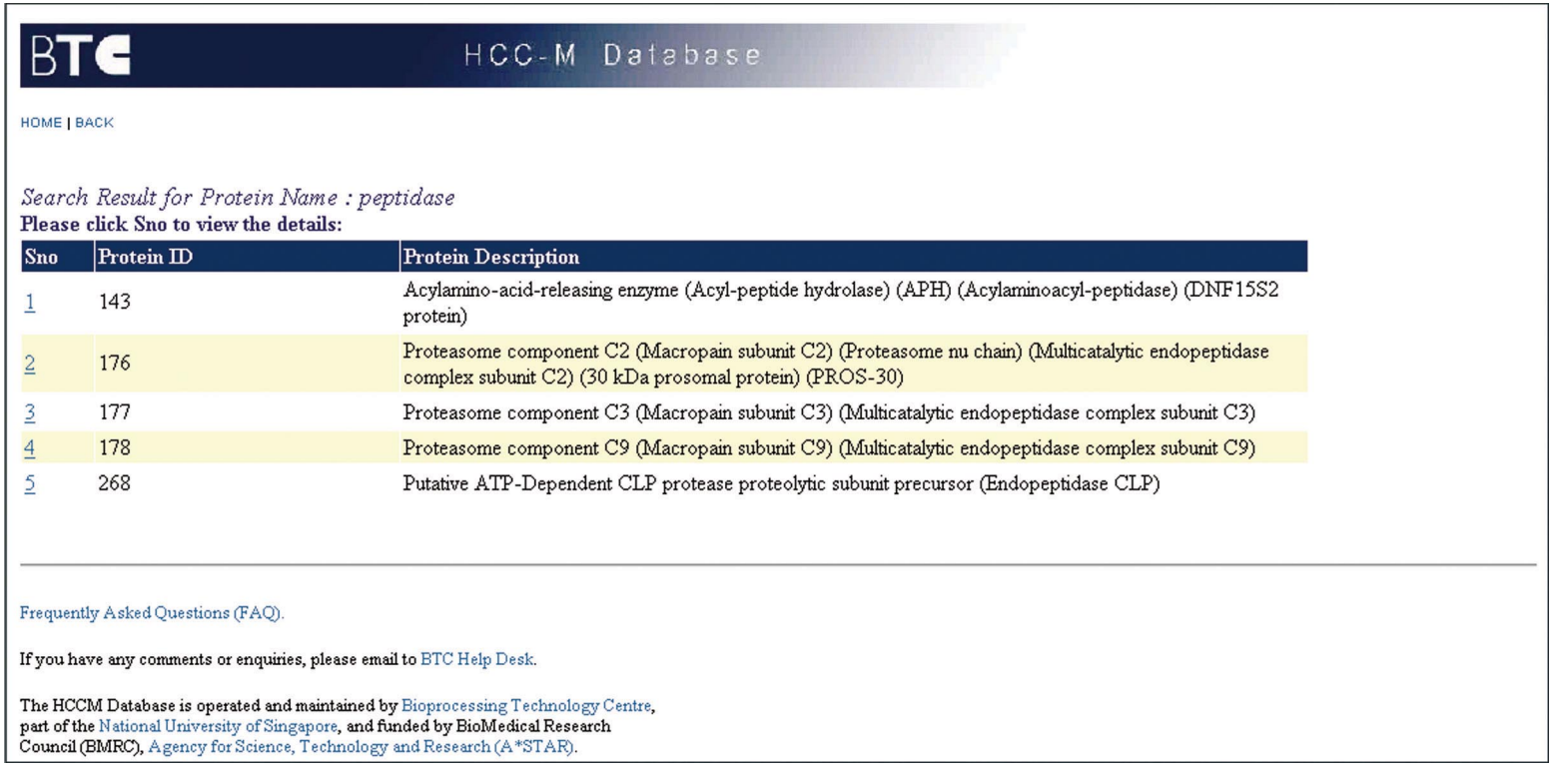

Fig. 4. Protein selection page.

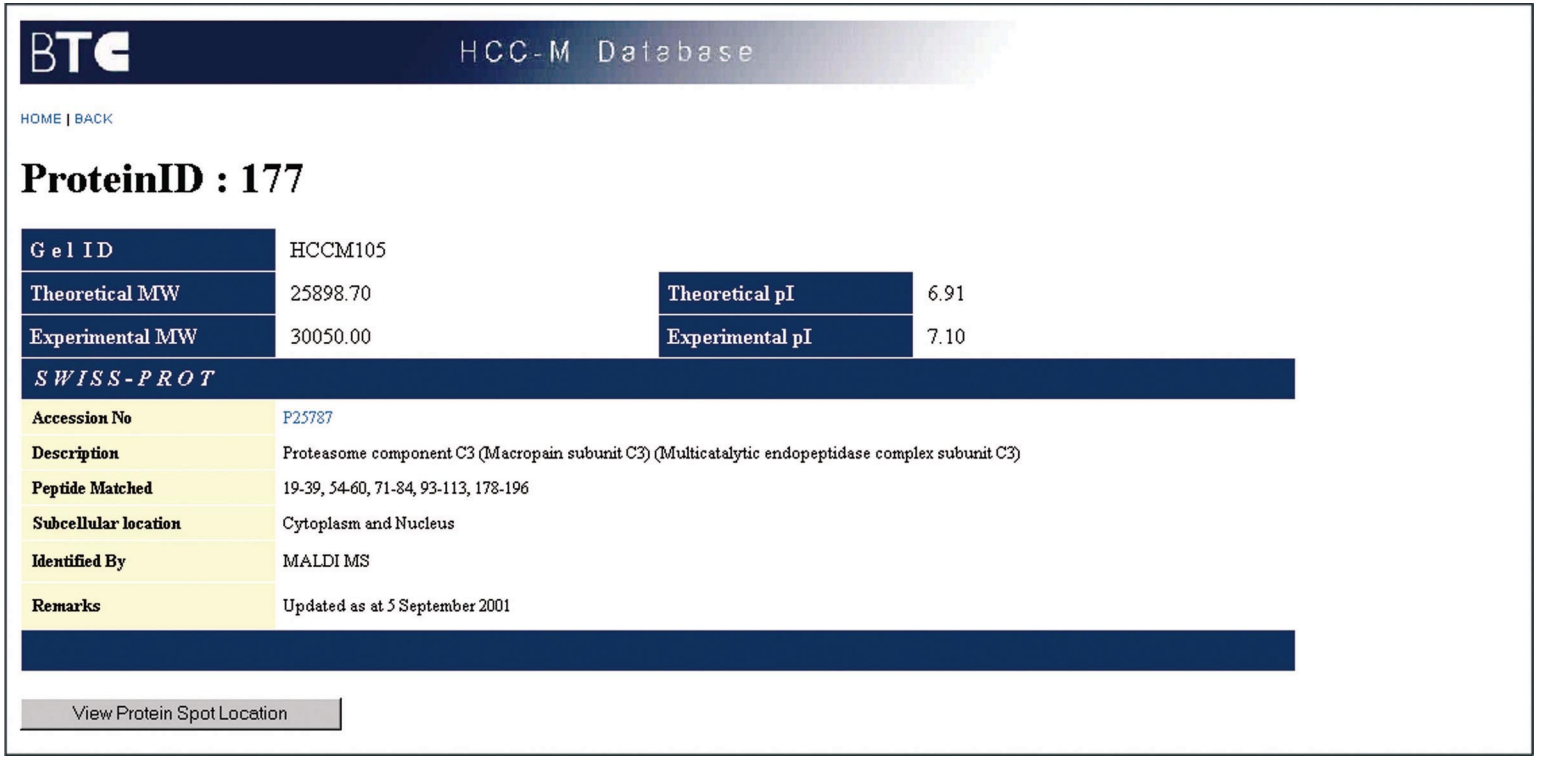

Fig. 5. Protein identity page.

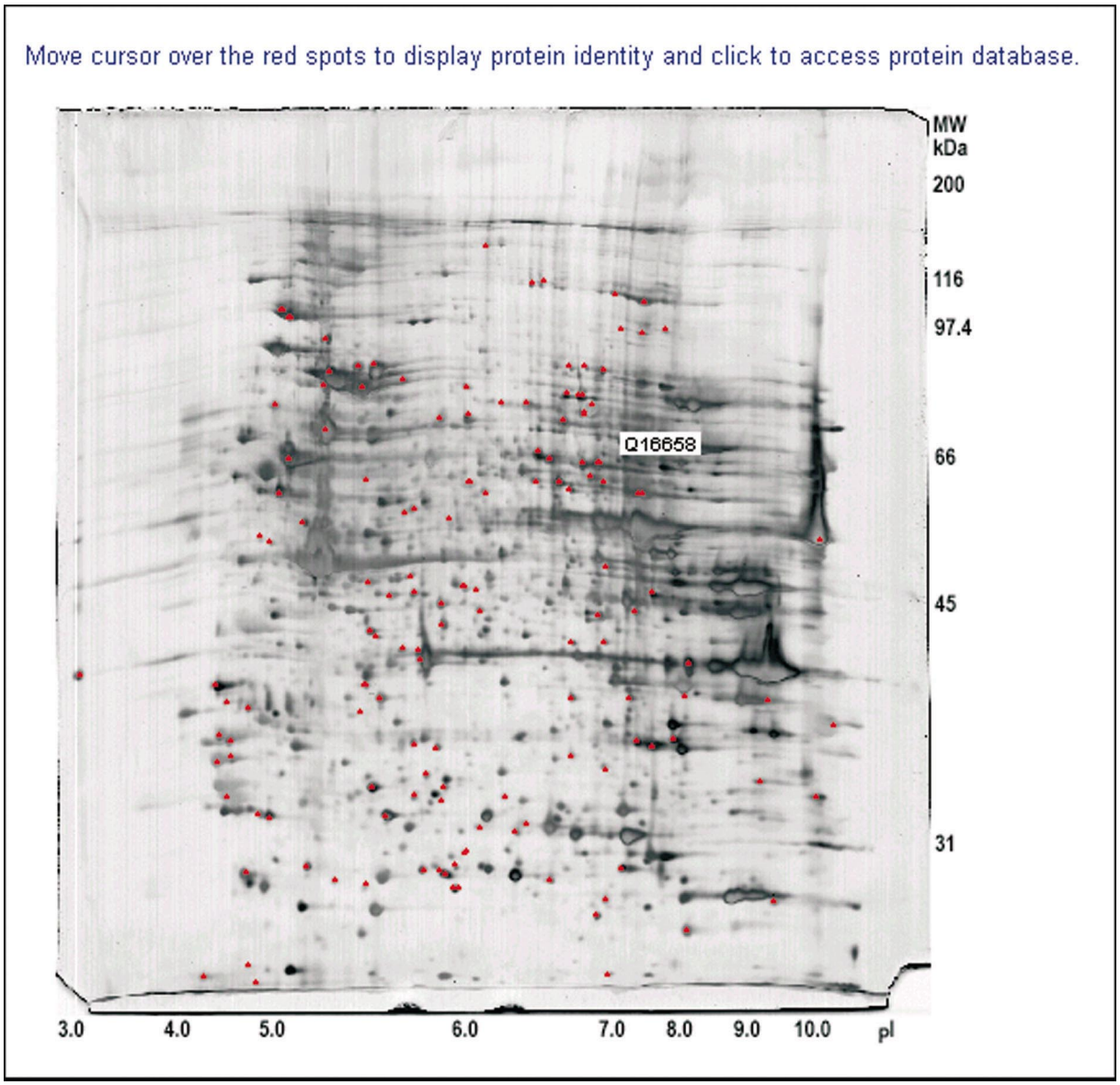

Fig. 6. HCCM074 image page.

accessed by clicking on the spot. The database is now freely accessible through the world wide web at http://proteome.btc.nus.edu.sg/hccm/.

4. Conclusions

As a result of the rapid development of proteomics, many proteome projects are currently underway. One of the major goals in this endeavour is to establish a protein database for the tissue, cell or model organism of interest that is accessible on the world wide web [10,19,20]. This will serve as a useful resource for scientists working in the same area of research. Thus, the establishment of the 2-DE proteome database of the HCC cell line, HCC-M, will be a useful repository of information for HCC. This would definitely facilitate the rapid identifica-

tion of novel diagnostic and therapeutic markers for HCC, which is an important first step towards the early diagnosis and treatment of this cancer.

5. Nomenclature

HCC or hepatoma	hepatocellular carcinoma
HBV	hepatitis B virus
HCV	hepatitis C virus
2-DE	two-dimensional electrophoresis
MALDI-TOF MS	matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry
nESI-MS–MS	nanoelectrospray ionisation tandem MS
DMEM	Dulbelcco's modified Eagle medium
FCS	foetal calf serum
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate
PMSF	phenylmethylsulphonyl fluoride
IEF	isoelectric focusing
IPG	immobilised pH gradient
DTT	dithiothreitol
SDS–PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis
IAA	iodoacetamide
ACN	acetonitrile
TFA	trifluoroacetic acid

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