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Nuclear Matrix Protein Expressions in Hepatocytes of Normal and Cirrhotic Rat Livers Under Normal and Regenerating Conditions

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Abstract We explored the feasibility of studying nuclear matrix protein (NMP) expressions of the hepatocytes in normal and cirrhotic rat livers with liver regeneration after partial hepatectomy. Sixteen Wistar healthy rats were studied with experimental liver regeneration and/or liver cirrhosis. Two-dimensional (2-D) gel electrophoresis was used to generate these NMP compositions from these rat liver samples. Several antibodies against cytokeratin, vimentin, actin, B23, HNF4alpha, and heat shock protein 70 were used for identification by Western blot. Totally, 41 strongly stained protein spots were characterized on the 2-D gels. Thirty-four protein spots were detected in all of these rat livers, of which, cytokeratin, vimentin, actin, HNF4alpha, and heat shock protein 70 were identified. B23 was detected in the regenerated livers. Three protein spots (s33, s34, and s35) were detectable only in NMP preparation extracted from the regenerating rat livers after hepatectomy. Another three protein spots (s36, s37, and s38) were detectable only in NMP preparation extracted from thioacetamide-induced cirrhotic rat livers. Under these conditions including experimental liver regeneration and/or liver cirrhosis, Over thirty higher abundance NMPs of hepatocytes were consistently expressed and considered as common and basic NMPs. Some of the NMPs are specific for liver regeneration and may play a critical role in cell proliferation and cell cycle, and some are specific for liver cirrhosis. J. Cell. Biochem. 91: 1269–1279, 2004.

Key words: nuclear matrix proteins; liver regeneration; partial hepatectomy; thioacetamide; liver cirrhosis; two-dimensional electrophoresis

Previous investigators studied protein expressions of hepatocytes in health and non-health states using two-dimensional (2-D) gel electrophoresis, most of them are consistently matched, and the different parts of protein expressions in both states are very valuable clue to probe gene expression effects in the states of liver diseases, physiological reaction, drug effects, and etc. Recently, a database of the rat liver cells as an in vivo biological system was developed to explore gene expression effects involving regulation of cholesterol synthesis by cholesterol-lowering drugs and a

Abbreviations used: PHx, partial hepatectomy; 2-D, two-dimensional; NM, nuclear matrix; NMPs, nuclear matrix proteins.

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high-cholesterol diet using 2-D gel electrophoresis [Anderson et al., 1991, 1995]. The liver protein patterns of male and female Wistar rats were investigated and sex-related proteins were detected [Steiner et al., 1995].

Nuclear matrix proteins (NMPs) are the nonhistone proteins, which contain nuclear matrix (NM) following nuclease, salt, and detergent extraction of isolated cell nuclei [Berezney et al., 1996]. Despite the high complexity, NMPs can be classified into at least two major types: (1) those which are commonly found in a variety of eukaryotic cells. (2) Those which are specific, such as cell-, tissue-, cancer-, mitotic-, and differentiation-specific NMPs. Several studies reported on 25 common NMPs from different human cell lines [Mattern et al., 1997], 15 minimal matrix proteins from rat, mouse tissues, and cell lines [Stuurman et al., 1990], and 102 NMPs from rat liver tissues [Korosec et al., 1997], under normal physiological condition. The common cytoskeletal proteins such as cytokeratin, actin, and vimentin were also found in the nuclear matrix preparation as reported previously [Korosec et al., 1997; Mattern et al., 1997; Fey and Penman, 1988]. In the 1990s, more researchers attempted to look for the NMPs specific for cancer. Four tumor-specific proteins were observed in ten out of ten-breast cancinoma and none out of the ten adjacent normal tissue samples [Khanuja et al., 1993].

The rat liver regeneration model is based on the concept that after partial hepatectomy (PHx) on either healthy rats or rat with liver cirrhosis, the hepatocytes proliferate compensatively and the livers regenerate. The liver regeneration model on healthy rat is a good in vivo model for the study of cell cycle and cell proliferation. [Rininger et al., 1997] reported alteration of several cell-cycle protein expressions following partial hepatectomy and drug-induced hepatic cell proliferation in rats. The liver regeneration model on the experimental rat with liver cirrhosis has always been used in clinical investigation for drug effect or selection of surgical operational procedures. Using the model here, the NMP expressions of hepatocytes in the disease and regenerating states were further compared.

At present study, we explored the feasibility of studying NMP expressions of the hepatocytes in normal and cirrhotic rat livers with liver regeneration after partial hepatectomy by nuclear matrix extraction and 2-D electrophoresis. Some of the NMPs were identified by specific antibodies and Western blot.

MATERIALS AND METHODS

Normal and Regenerating Rat Livers

Animals. Ten Wistar male rats of 300–500 g were used. All the rats were kept on a controlled lighting schedule with a 12-h dark period. Food and water were available ad labium but prior to experiments the rats were fasted overnight. The rats were prepared for experimental liver regeneration post operation.

Partial hepatectomies. Ten of the normal rats were used for PHx under either anesthesia according to the method of Higgin and Anderson [1931]. Briefly, the large median and the left lateral lobes were surgically excised. The resected liver tissues were used as controls. In this procedure, 65–75% of the liver were removed, leaving the right lateral and the small caudate or spigelian, lobes. Seven of the rats with PHx survived. The regenerating liver tissues were obtained at post-operation days 1, 2, 3, 4, and 7 after PHx.

Thioacetamide Induced Cirrhotic Rats Livers and Thioacetamide Induced Regenerating Cirrhotic Rat Liver

Animals. Six Wistar male rats, 150–250 g were used. All the rats were 2 months old and administered with the drug thioacetamide (C2N5SN, FW75.13, Sigma–Aldrich, St. Louis, MO), three times (300 mg TA/kg) a week for 2 months [Yin et al., 2000]. Two were scarified to obtained thioacetamide induced cirrhotic rat liver tissues that were confirmed by two pathologists and four for regeneration after PHx as below.

Partial hepatectomies. PHx were performed on four rats as described above [Yin et al., 2000]. The regenerating cirrhotic liver tissues were obtained at post-operation days 1, 2, 4, and 7 after PHx.

Hepatocyte Isolation

Mechanical methods including homogenize, steel mesh (200 μm) filter, and centrifugation at 100*g* in triplicate were used with minor modification in the isolation of hepatocytes as reported previously [Park et al., 1994].

NMP Extraction

The NMPs were isolated by the method as described by Fey and Penman [1988]. The liver

cells were extracted in cytoskeleton buffer containing 100 mM KCl, 3 mM $MgCl_2$, 1 mM EGTA, 1 mM PMSF, 10 mM PIPES pH 6.8, 300 mM sucrose, and 0.5% Triton X-100 for 15 min at 4°C. The chromatin was removed by incubation of the pellet in DNase I (100 μg/ml) and RNase A (100 μg/ml) in a buffer solution in the presence of 50 mM NaCl instead of KCl with protease inhibitor for 30 min at room temperature and terminated by the addition of cold ammonium sulfate to a final concentration for 0.25 M. The remaining pellet fraction, which contained the nuclear matrix and intermediate filament proteins, was solubilized in the disassembly buffer containing 8 M urea, protease inhibitors and 1% (v/v) β-mercaptoethanol and dialyzed for 16 h at 4°C against 1,000 volume of assembly buffer (0.15 M KCl, 25 mM imidazole hydrochloride pH 7.1, 5 mM $MgCl_2$, 0.125 mM EGTA, 2 mM dithiothreitol, 0.2 mM PMSF). Intermediate filaments were removed by ultracentrifugation at 150,000*g* for 90 min, leaving the NMPs in the supernatant fraction. The Dc Protein Assay Kit (BioRad, Hercules, CA) was used to determine the NMP concentration with bovine serum albumin as the standard marker. The proteins were then precipitated in five volumes of absolute ethanol. For 2-D gel electrophoresis, the NMPs were re-dissolved in a sample buffer consisting of 9 M urea, 2% Triton X-100, 2% ampholytes, and 5% (v/v)-mercaptoethanol.

2-D Gel Electrophoresis

High-resolution 2-D gel electrophoresis was carried out by using the Mini-PROTEAN II electrophoresis system (BioRad) [Yang et al., 1997; Yam et al., 1998; Jin et al., 2001]. Onehundred micrograms of NMPs were dissolved in the first dimensional gel made by 9.2 M urea, 4% acrylamide, 20% Triton X-100, 1.6% Bio-Lyte 5/7 ampholyte, 0.4% Bio-Lyte 3/10 ampholyte, 0.01% ammonium persulfate, and 0.1% TEMED, which were used for isoelectric focusing in 1.0 mm diameter IEF tubes with 7.5 cm long in steps at 500 V for 30 min, at 300 V for 12 h, and then at 500 V for 2 h. Gels were extruded and incubated in equilibration buffer consisting of 71 mM Tris-HCl, pH 6.8, 2.9% SDS, 0.003% bromphenol blue for 10 min, and then placed on top of 10% SDS–polyacylamide slab gels (80 × 60 × 1 mm) containing 4% acrylamide stacking gels (upper) and 10% acrylamide resolving gels (lower) for second-dimensional separation at 150 Vh. The electrophoresis was performed at 20°C in running buffer consisting of 192 mM glycine, 25 mM Tris-base, and 0.1% SDS at 150 V until the blue in color went down the bottom of the second dimensional gels. The gels were then silver stained [Rabilloud, 1992] or transblotted onto nitrocellulose membranes.

Image Analysis

Staining slab gels were digitized in gray light using Model GS-700 Imaging Densitometer (BioRad) with a resolution 42 μm (600 bpi). The digitized 600 bpi images were treated using Molecular analyst software (BioRad) and then saved as TIFF files, which were printed out by PowerPoint (Figs. 1–4). The raw images (TIFF images) were processed using BioRad® Phoretix 2-D Advanced software system to yield lists of spot position, shape, and density for each detected spots (Figs. 5 and 6). To enable automated comparison of protein patterns, individual spots were manually defined as anchors the images of each group livers, and a few of strongly-stained spots which always showed in different samples were selected for reference spots. The gels were grouped and matched to obtain composite images. For comparison of spot patterns, primarily the position, shape and size of the spots were taken into account. Coelectrophoresis experiments with selected pairs of samples were performed. By serial matching images, the common NMPs or specific NMPs were defined. For semi-quantitative estimation of protein concentrations [Korosec et al., 1997], the integrated optical intensities of individual spots were calculated and expressed in percentages of the total integrated spot intensities (Table I).

Western Blot Analysis

Antibodies. Cytokeratin 18 (DC-10, Santa Cruz, CA), vimentin (1:300, M0725, DAKO, Glostrup, Denmark), actin (1:200,C-11, Santa Cruz, CA), B23 (a gift of Dr. Chan P.K. and Dr. John Y.H. Chan [Yun et al., 2002, 2003]), HNF4alpha (1:500, Santa Cruz), and heat shock protein 70 (1:200, W27, Santa Cruz). The slab gels including SDS–PAGE gels and 2-DE gels were processed for Western blot analysis as previous method [Jin et al., 2001; Yun et al., 2002, 2003]. Briefly, the gels were first equilibrated and the proteins in the gels were transferred onto blotting membranes. After blocking,

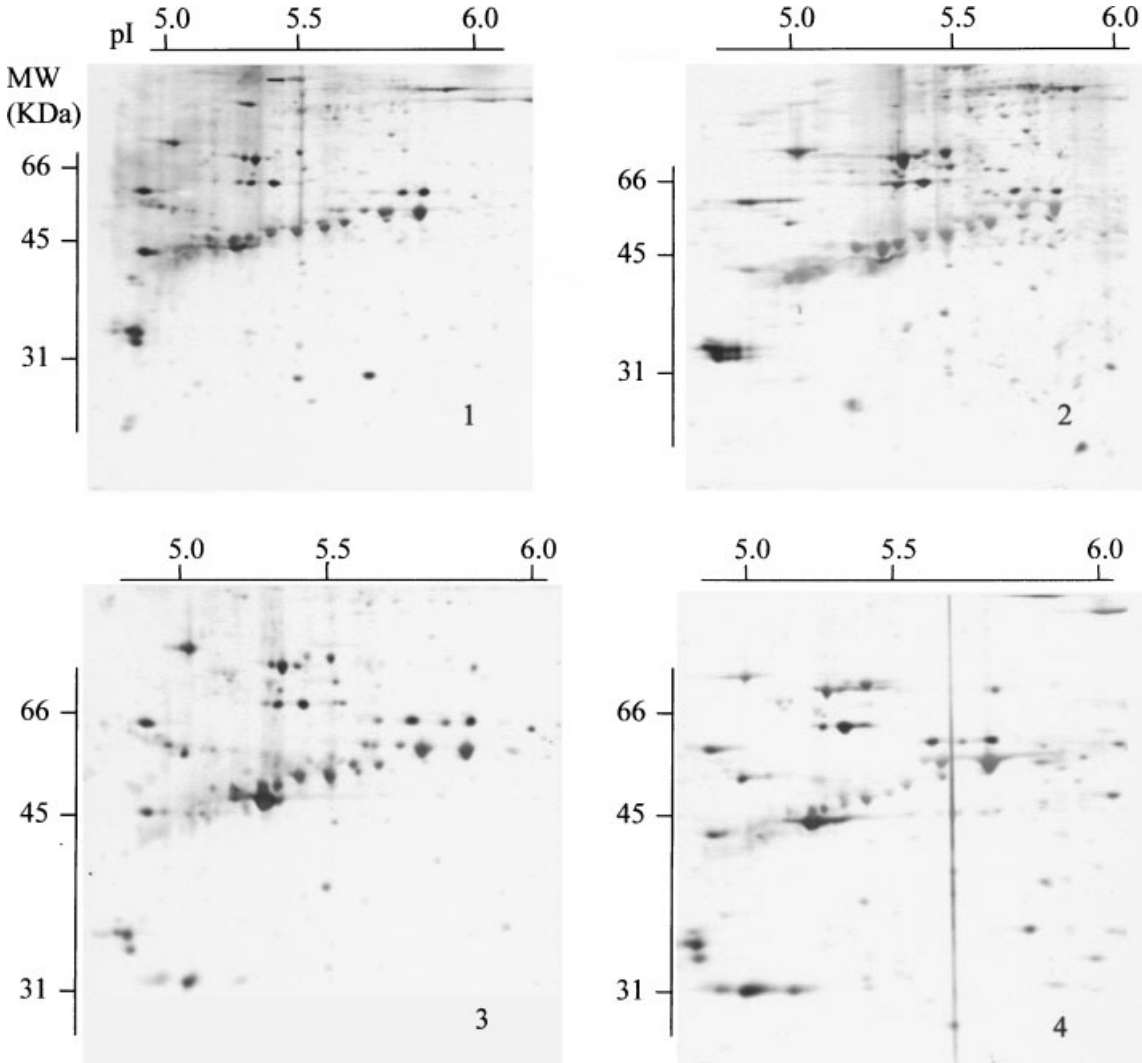

Fig. 1. Four representative images (TIFF image) of NMP compositions extracted from normal rat livers and regenerating rat livers at different time periods after partial hepatectomy (PHx) by 2-D electrophoresis. **Part 1**, normal rat liver; **part 2**, regenerating rat liver at post-operation day 1 after PHx; **part 3**, regenerating rat liver at post-operation day 3 after PHx; **part 4**, regenerating rat liver at post-operation day 7 after PHx. 2-D, two-dimensional; PHx, partial hepatectomy; MW, molecular weight.

the membranes were routinely incubated with mouse monoclonal antibody against B23, rabbit anti-mouse IgG (Amersham Life Science, Arlington Heights, IL) and horseradish peroxidase-conjugated mouse anti-rabbit IgG. The blots were then incubated with ECL kit (Amersham Life Science, NH) and developed for 3 min with X-ray film.

RESULTS

NMP patterns from rat livers with four different conditions below were isolated and analyzed by 2-D gel electrophoresis. Rat livers with four different conditions were obtained from: (1) normal rat livers ($n = 10$); (2) regenerating normal rat livers ($n = 10$) at post-operation days 1, 2, 3, 4, and 7 after PHx; (3) thioacethamide induced cirrhotic livers ($n = 6$); and (4) thioacethamide induced regenerating cirrhotic livers ($n = 4$) at post-operation days 1, 2, 4, and 7 after PHx. All the liver tissues were collected fresh and each of the NMP preparations was run in duplicate or triplicate.

Approximately 200 NMP spots on the 2-D gels (IEF) after silver staining were obtained from the above liver samples. Few of the spots in the IEF gels were stained relatively strong and were used to compare with different samples. Most of the spots were weakly stained and sometimes showed variation in the IEF gels

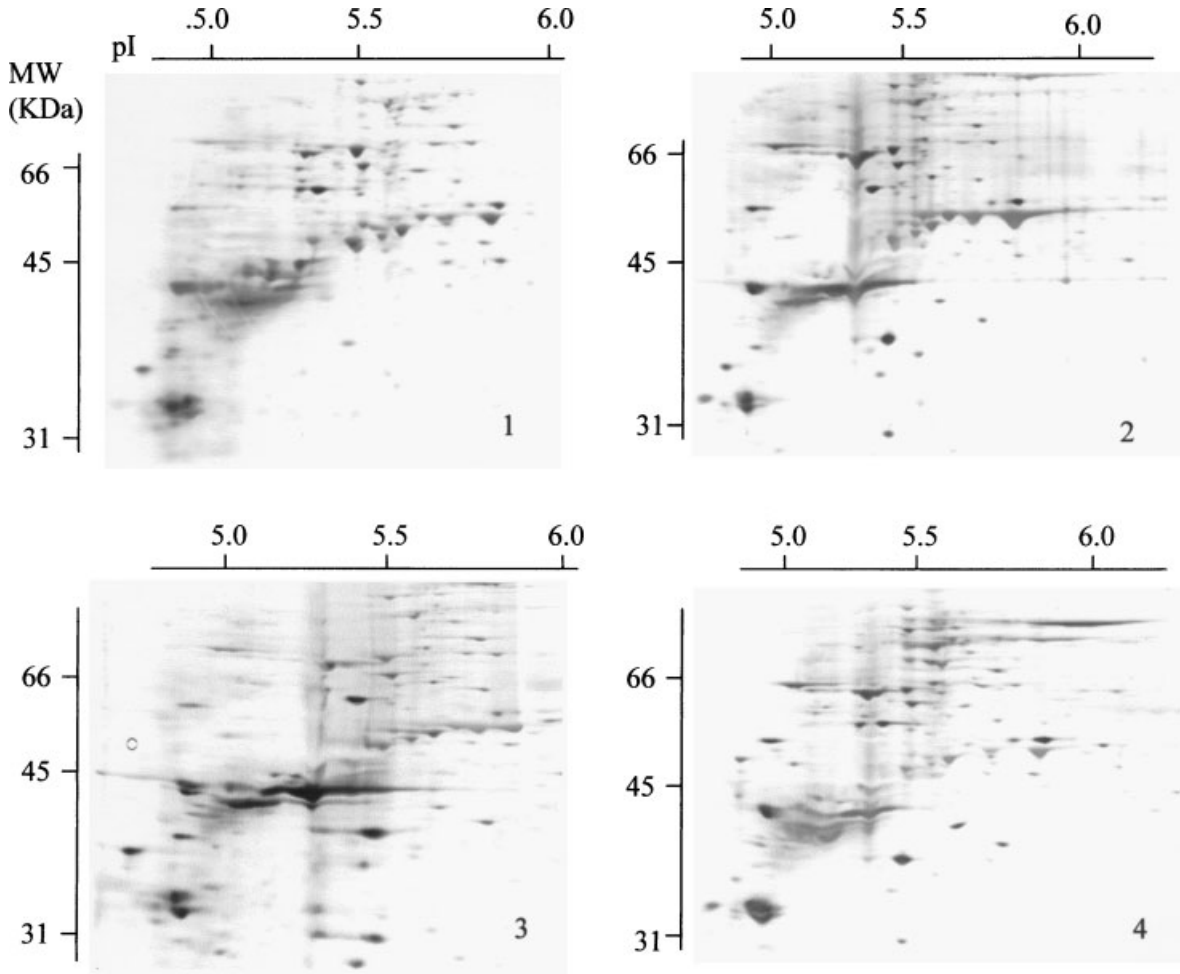

Fig. 2. Four representative images (TIFF images) of NMP composition extracted from thioacetamide induced cirrhotic livers and regenerating cirrhotic livers at different time periods after PHx by 2-D electrophoresis. **Part 1**, thioacetamide induced cirrhotic liver; **part 2**, regenerating cirrhotic liver at post-operation day 1 after PHx; **part 3**, regenerating cirrhotic liver at post-operation day 4 after PHx; **part 4**, regenerating cirrhotic liver at post-operation day 7 after PHx.

in different samples. The determination of the common or specific NMPs was based on matching and comparing the composite images characteristic for the relatively abundant NMPs of liver cells from different rat liver tissues. Protein spots that were found in each of the NMP patterns from the different liver tissues were defined as common NMPs and those that were detected only in a specific condition were considered as specific proteins.

Forty-one strongly stained protein spots were characterized on the 2-D gels. Thirty-four relatively strongly stained spots appeared in the NMP preparation from both the normal rat livers and the normal regenerating rat livers after PHx. These spots were also detected in the NMP preparation either from the experimental cirrhotic rat livers or the regenerating cirrhotic rat livers after PHx (Figs. 1, 2, and 5). The molecular weights of these spots ranged from 30 to 80 kDa and pI ranged from 4.5 to 7.5 (Table I). Semi-quantitative results of each spot among different rat liver samples could be shown in Table I based on the protein concentration of protein spots estimated by the 2-D analysis software. In the regenerating livers after PHx, some of the 34 spots had quantitative differences when compared with the normal livers or the regenerating livers at different time periods after PHx, as also observed in the thioacetamide induced cirrhotic livers with regeneration after PHx.

Four protein spots present in all of the nuclear matrix preparation of rat livers were identifi-

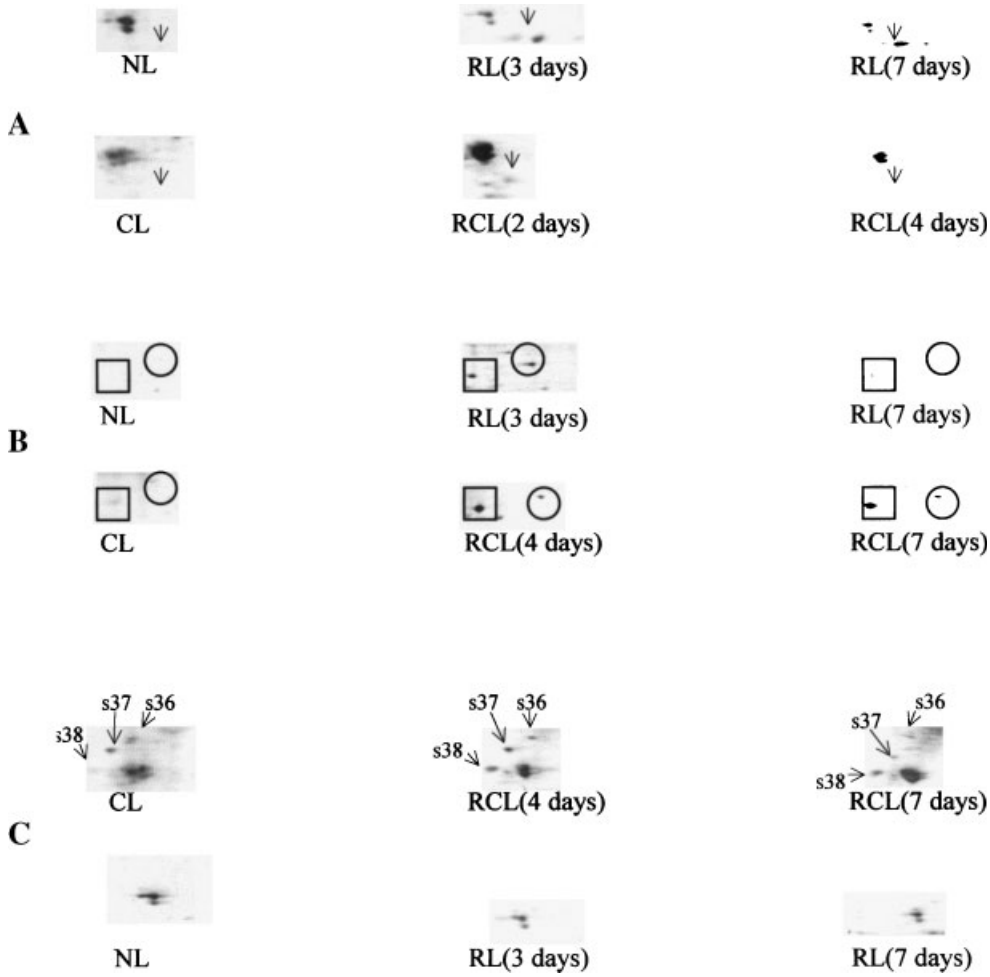

Fig. 3. Specific NMPs in the 2-D gels extracted from rat livers. **A**: Showed spot s33 (indicated in arrows) absent or weakly present in the non-regenerating rat livers (NL and CL) and present in the regenerating livers (RL and RCL); (**B**) showed spot s34 (encircled in squares) and s35 (encircled in circles) absent or weakly present in the non-regenerating livers (NL and CL) and present in the regenerating livers (RL and RCL); (**C**) showed three spots (spot s36, s37, and s38) present in the cirrhotic livers (CL and RCL) and absent in the livers without drug treatment (NL and RL). NL, normal liver; RL, regenerating liver at different time periods (in brackets) after PHx; CL, thioacetamide induced cirrhotic liver; RCL, regenerating cirrhotic liver at different time periods (in brackets) after PHx.

ed by immunoblotting as actin, HNF4 alpha, vimentin, and cytokeratin (data to be published). Previous studies have shown that cytoskeletal structures co-isolate with nuclear matrix preparation. The intermediate filaments were highly associated with the nuclear lamina [Verheijen et al., 1986; Penman, 1995; Mattern et al., 1996]. Other common NMPs such as PDI, ER60, HSP70, F1 ATPase, and NMP238 were tentatively assigned and identified by their molecular weight and electronic point as compared to the database [Anderson et al., 1995] and reference [Holzmann et al., 1998].

Four protein spots were detected in the regenerative livers. B23 were identified with Western blot analysis [Yun et al., 2003]. Three protein spots (Fig. 3; s33, s34, and s35) were detected only in the NMPs from the regenerating rat livers which include the normal regenerating and thioacetamide induced regenerating cirrhotic livers after PHx. These three spots were absent in normal and thioacetamide induced cirrhotic liver (Fig. 4, Table I). Spot S33 was intensified in the normal regenerating liver but it was relatively low among the regenerating cirrhotic livers. Spot S34 and spot S35 were relatively low abundance in the normal regenerating liver but they were present in significantly higher amounts in the regenerating cirrhotic livers. These four

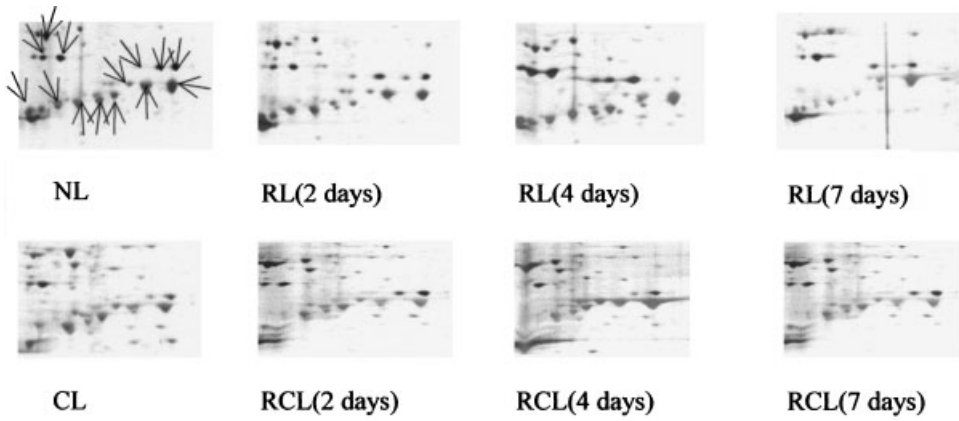

Fig. 4. Parts of common NMPs (indicated in arrows) showed in all the 2-D gels extracted from different liver tissues including normal livers (NL), regenerating livers at different time periods after PHx (RL at 2 days, RL at 4 days, RL at 7 days), thioacetamide induced cirrhotic livers (CL), and regenerating cirrhotic livers at different time periods after PHx (RCL at 2 days, RCL at 4 days and RCL at 7 days).

peptides were identified as possible factors associated with liver cell proliferation and differentiation.

Three spots (Fig. 4, Table I, Spot s36, s37, and s38) were detected in all of the cirrhotic rat livers including the regenerating cirrhotic liver samples. However, it was absent in the normal livers including the normal regenerating rat livers. Therefore, three peptides were identified as factors associated with the thioacetamide induced cirrhotic livers.

DISCUSSION

Several studies on common NMPs reported were all from liver with normal physiological condition [Stuurman et al., 1990; Korosec et al., 1997; Mattern et al., 1997]. In this study, common NMPs of rat livers were identified and defined by their molecular weights and isoelectric points in four different conditions. Thirty-five relatively strongly stained spots appeared in the NMP preparation either from

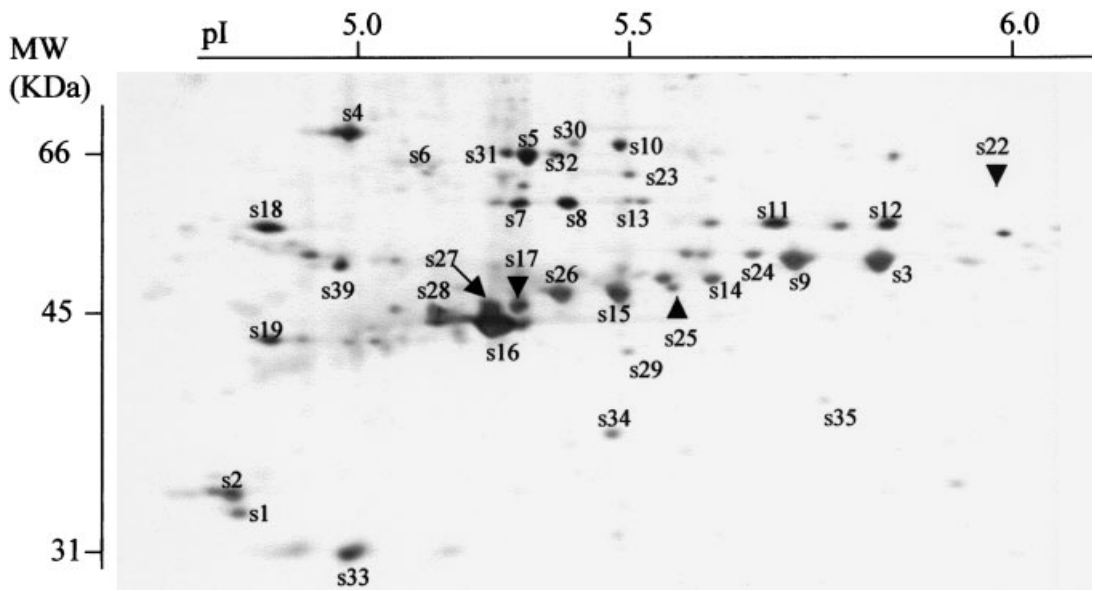

Fig. 5. Computed image of NMP composition extracted from regenerating livers at day 2 after PHx by 2-D electrophoresis. The spot numbers were correspondent to parts of those spots listed in table. A few of the spots were absent from the image.

TABLE I. Nuclear Matrix Proteins (NMPs) of Rat Livers With Normal, Cirrhotic, Non-Regenerating, and Regenerating Conditions

Spots			Rat livers (%)[a]				
No.	MW	pI	NL	RL	CL	RCL	Identification
S1	33.6	4.75	1.3	1.8	2.9	3.1	
S2	34.7	4.8	0.9	2.3	3.6	2.5	
S3	50.5	5.8	2.5	5.4	1.9	3.5	rNMP238 [Holzmann et al., 1998]
S4	71	5	1.6	2.2	0.9	1.8	
S5	67	5.4	1.9	2.5	2.1	1.5	
S6	63.7	5.18	0.2	0.3	0.6	0.2	
S7	60.8	5.35	0.6	0.4	1.3	1.0	
S8	60.8	5.45	1.0	1.2	1.0	1.3	
S9	50.5	5.67	2.3	4.1	1.2	2.1	Cytokeratin [Anderson et al., 1995], I
S10	69	5.54	0.7	0.9	0.4	0.8	HSP70 [Anderson et al., 1995]
S11	57	5.63	1.1	0.7	0.6	1.2	Protein disulfide isomerase [Anderson et al., 1995]
S12	57	5.85	0.6	0.7	1.0	1.3	ER60 [Anderson et al., 1995]
S13	60.8	5.56	0.6	0.7	0.6	0.5	
S14	48.5	5.6	1.5	1.6	0.9	0.6	
S15	47	5.52	3.1	3.6	3.1	2.3	
S16	43	5.3	4.5	8.4	6.2	5.3	
S17	45	5.35	2.6	2.2	2.0	3.5	
S18	54.6	4.8	2.1	1.1	0.6	0.6	Vimentin [Anderson et al., 1995], I
S19	43	4.8	3.0	3.3	2.0	2.8	
S20	40	7.2	0.9	1.4	1.2	2.6	
S21	44	6.7	0.8	0.6	0.3	0.9	
S22	61.7	5.9	0.2	0.3	0.1	0.9	
S23	63	5.55	1.1	0.3	0.5	1.2	
S24	50.5	5.62	0.4	0.5	0.5	0.6	
S25	48	5.56	0.5	0.6	0.8	1.2	
S26	47	5.44	1.6	2.6	1.3	1.2	
S27	45	5.3	2.5	2.7	2.6	3.3	Actin [Anderson et al., 1995], I
S28	45	5.19	3.6	3.4	5.6	3.9	
S29	42	5.56	0.3	0.3	0.6	0.9	
S30	69	5.46	0.6	0.2	0.6	0.8	
S31	67	5.35	1.2	0.6	0.9	1.2	
S32	67	5.43	0.8	0.6	0.7	1.4	
S33	31	5.06	0.0	1.7	0.0	0.8	
S34	38	5.54	0.0	0.4	0.0	1.2	
S35	40	5.7	0.0	0.2	0.0	1.6	
S36	38	4.8	0.0	0.0	0.7	0.6	
S37	36.5	4.5	0.0	0.0	0.6	0.8	
S38	34.7	4.3	0.0	0.0	1.0	0.9	
S39	50	5	0.8	0.6	1.5	1.7	F1 ATPase b [Anderson et al., 1995]
S40	40	5.1	0.0	1.6	1.3	1.5	B23 [Yun et al., 2002, 2003]
S41	50	5.1	0.4	0.6	0.6	0.5	HNF4alpha, I

NL, normal livers; RL, regenerating livers at different time periods (1, 2, 3, 4, and 7 days) after PHx; CL, thioacetamide induced cirrhotic livers; RCL, regenerating cirrhostic livers at different time periods (1, 2, 4, and 7 days) after PHx; I, identified by immunostaining.
[a]The percentage of proteins from four different rat livers was calculated on the volumes of each spots listed by two-dimensional (2-D) analysis software.

both the normal rat livers and the normal regenerating rat livers after PHx or from the experimental cirrhotic rat livers and the regenerating cirrhotic rat livers after PHx. The study analyzed common NMPs from rat livers with either normal physiological or abnormal condition. The findings indicated that the common NMPs maintained a relatively high quantity in cell cycle of liver cells in vivo in liver regeneration after PHx or in abnormal condition of liver cells such as drug induced cirrhosis. It is reasonable to think that these common NMPs played an essential role in the organization of the nucleus.

The common cytoskeletal proteins such as cytokeratin, actin, and vimentin were also found in the nuclear matrix preparation as reported previously [Fey et al., 1988; Korosec et al., 1997; Mattern et al., 1997]. In analogy to what was known for the cytoplasmic pendant of the nuclear matrix, the cytoskeleton was likely to have an important structural role in the nucleus. Some of the other common NMPs were also identified by their isoelectric point and molecular weight as compared to those reported in the database [Anderson et al., 1995] and articles [Holzmann et al., 1998]. NMP238, the recently described ubiquitously occurring NMP and relating to subunits of T-complex protein-1 (TCP-1), was an evolutionary conserved protein potentially mediating the assembly and interaction of nuclear proteins [Holzmann et al.,

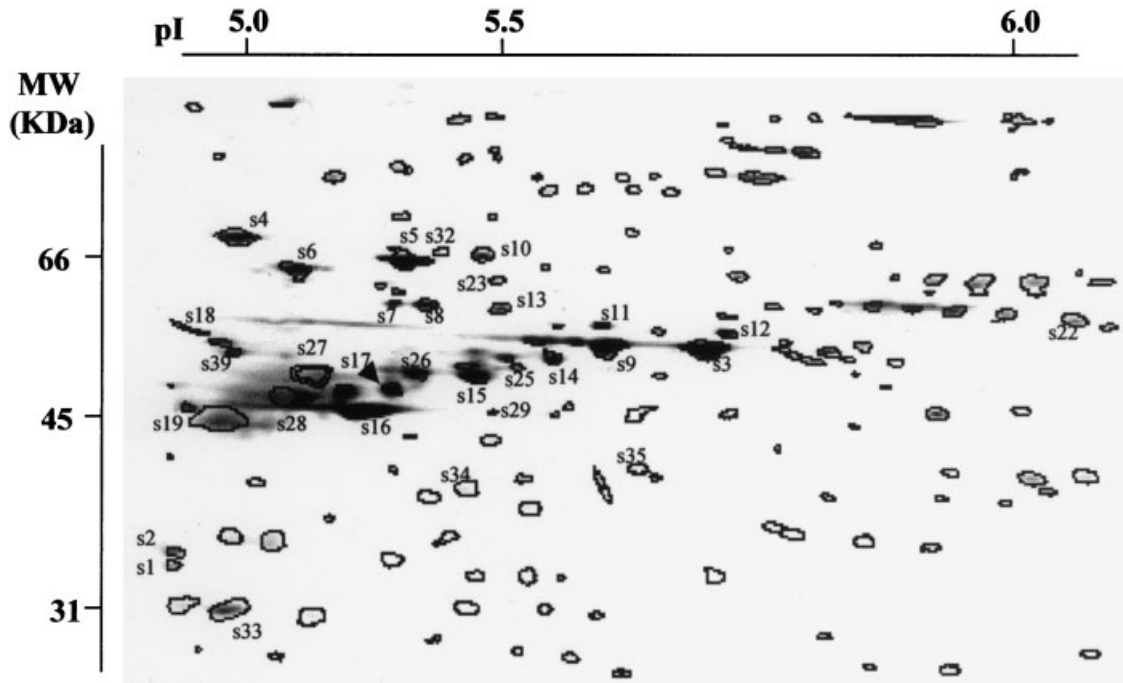

Fig. 6. Computed image of NMP composition extracted from regenerating livers at day 2 post-operation after PHx by 2-D electrophoresis. The spot numbers were correspondent to those spots listed in Table I. Some of the spots were absent from the image.

1998]. PDI, HSP70, and ER-60 were also identified as NMPs [Gerner et al., 1999]. PDI was an abundant ubiquitous protein of endoplasmic reticulums that catalyzed the oxidation, reduction, and isomerization of protein disulfides and participated in the regulation of reception function, cell–cell interaction, gene expression, and actin filament polymerization. ER-60 was a chaperon with thiol-dependent reductase activity involved in MHC class I assembly and HSP70 was one of the heat shock protein families that played a role in the protection of protein traffic and secretion, as well as in cytoskeleton organization. F1-ATPase was the portion of the ATP synthetase that contained the catalytic and regulatory nucleotide binding sites and catalyzed the synthesis of ATP for cellular function. HNF4 alpha were first reported to find in the NMP preparation of heapteocytes (data to be published). These proteins identified in the nuclear matrix indicated that they may have a structural role in the nucleus.

Apart from the common proteins, this study also demonstrated some differences in the minor protein composition of nuclear matrices isolated from different rat liver tissues. Several investigators have reported the presence of cell-type-specific and cell-differentiation-specific proteins in the nuclear matrix preparations of tumor-derived and normal cells [Fey et al., 1988; Khanuja et al., 1993; Partin et al., 1993; Keesee et al., 1994; Getzenberg et al., 1996] and thioacetamide induced proteins [Dyroff and Neal, 1981]. In this study, the four peptides or proteins of rat liver cells present or intensified in the regenerating rat livers after PHx were likely to be involved in the cell cycle and differentiation in regeneration of liver cells, B23 was demonstrated to be upregulated at protein expression level in the regenerative livers [Yun et al., 2003]. Another three protein spots just tentatively assigned by their molecular weights and isoelectric points will be identified by microsequecing and mass spectroscopy.

Another three proteins of rat liver distinctly differed from the above three proteins were found in the nuclear matrix preparation isolated from thioacetamide induced cirrhotic rat livers. It is unknown that the expression of these three proteins were drug or cirrhosis related. Several previous studies showed the alteration of hepatic proteins induced by thioacetamide exposure [Dyroff and Neal, 1981; Krajewska et al., 1993; Witzmann et al., 1996], however, the finding in this study was slightly

different from those previously. The three proteins were probably induced by the process of liver cirrhosis and not by the drug thioacetamide. To date, there has been no report on specific proteins for liver cells from cirrhosis. In this study, new NMP spots have been identified.

In addition, the nuclear matrix preparation protocol used a dialysis of a solubilize nuclear matrix-intermediate filament protein fraction against a reassembly buffer to remove cytofilament proteins selectively [Fey et al., 1988], which was universally applied in the NMP extraction from culture cell lines or from tissues [Keesee et al., 1994; Getzenberg et al., 1996; Yang et al., 1997; Yam et al., 1998; Jin et al., 2002]. In this study, the NMPs extracted from rat livers with four different conditions including normal, cirrhotic, and regenerating livers were investigated by using the same extraction protocol. Most of the strongly silver-stained peptides or proteins extracted from different rat livers commonly appeared in the NMP preparations. There are also differences observed in the NMP compositions. The similarity and differences are both reproducible. The NMPs extracted from the different liver tissues were resolved well and reproducible by IEF technique. Furthermore, acid proteins in the protein complexes were also well resolved by using IEF. Therefore, IEF was used to resolve and analyze the common and specific peptides of nuclear matrix preparation in this study.

Present study leads to several important issues that need to study later. Further study to identify the interesting proteins and elucidate the function and value of these proteins in the nucleus should be forthcoming.

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