Proteomic Profiling and Protein Identification by 2-DE gel electrophoresis combined with MALDI-TOF Mass Spectrometry in Rat hepatocyte Nucleus

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Abstract: More and more proteomics research focusing on nucleoprotein. In this article, the proteomic profiling of Rat hepatocyte nucleus was showed by 2-D PAGE and a total of 204 kinds of nucleoproteins were identified by MALDI-TOF Mass Spectrometry. Addition to housekeeping protein, low kurtosis transcription factors were enriched and identified too. Ingenuity Pathway Analysis (IPA) showed that these nucleoproteins were involved in the rat liver cell cycle regulation, DNA replication, recombination and damage repair, cell assembly and organization, cell development, nervous system development and function, cancer, cardiovascular system development and function, cell death and survival, cell morphology, Cell-To-Cell signaling and interaction. These results can provide more information for a comprehensive understanding to the function of nucleus; on the other hand, the results lay the foundation for the analysis of nucleoproteins expression varieties and help to look for the biomarker of disease diagnosis and therapies.

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1. Introduction

Proteomics has emerged as a powerful tool towards a deep understanding of human and mammalian disease. Compared to genomics, proteomics provides not only information at a mechanistic level but can also capture changes in protein activity measured as post-translational modifications (Fernando et al., 2013; Tung et al., 2013). In fact the transcriptome does not account for the post-transcriptional and post-translational regulation of protein expression. Most studies reveal a poor correlation between protein expression and changes in transcript level (Pandey and Mann, 2000). Proteomics becoming an effective method for the global recognition and identification of proteins expressed in cell or tissue, and is able to identify the expression pattern and functional modes of the proteins. Proteomics mainly include three types: expression proteomics, structural proteomics and functional proteomics (Schmid, 2002; Patterson and Aebersold, 2003; Aggarwal and Lee, 2003). In recent years, proteomics based on two-dimensional electrophoresis has walked into the subcellular level. Combined subcellular fractionation and multi-stage extraction sample preparation, Organelle proteome has become a new hot spot in proteomics research (Duclos and Desjardins, 2011; Paulo et al., 2013; Mulvey et al., 2013).

As the largest and most important organelles in eukaryotic cells, the nucleus is the center of genetic control and metabolic regulation in the cell (Misteli and Spector, 1998; Dundr and Misteli, 2001). As a direct reflection of the functional genes, there are numerous functional proteins in the nucleus, which have been implicated in essential processes such as transcription and splicing. They are the most important part of the cellular structures and play important role during life activities in the cell (Lamond and Earnshaw, 1998; Lewis and Tollervey, 2000). Proteins of the nucleus are dynamic, temporal and adjustable. Proteins involved in diverse nuclear processes move rapidly throughout the entire nucleus and rapidly associate and dissociate with nuclear compartments (Phair and Misteli, 2000), some involved in transcriptional regulation, some involved in the transport of nuclear and cytoplasm. Nucleus proteomics research has started more than a decade (Jung et al, 2000). Nuclear proteomics analysis found a variety of diseases are associated with the nucleoprotein (Shakib et al., 2005; Qazi et al., 2011).

Here, we performed a detailed proteomic analysis of rat hepatocyte nuclei, establishing and optimizing the nucleoprotein proteomics technology system based on two-dimensional electrophoresis combined with MALDI-TOF mass spectrometry. IPA (Ingenuity Pathway Analysis) software was used to analysis the physiological functions of nucleoprotein. Our analysis provides unprecedented insight into the rat

hepatocyte nuclei and will be of great benefit to understand the structure and function of nucleus.

2. Material and Methods

2.1 isolation and immunochemical identification of hepatocytes

Hepatocyte isolation from rat livers is carried out with a "two-step" collagenase procedure (Vondran et al., 2008). The procedure involves the initial perfusion of the liver with a warm (37 °C) divalent ion-free, EGTA-containing, isotonic buffer (Step 1) to remove blood and to loosen cell-cell junctions, followed by perfusion with a warm, isotonic, collagenase solution (Step 2) to dissociate the liver parenchyma into single cells. After digestion, the cells are harvested by low-speed centrifugation. A density gradient of 60% Percoll is used to enrich hepatocytes.

A few purified hepatocytes were fixed with 10% formaldehyde for 30 min, and then smeared onto glass slides. When the cells dried on glass slides, microwave antigen retrieval was performed. The sections were incubated with 1:200 dilution (*V/V*) of ALB antibody (Santa, USA) overnight at 4℃ and then incubated with 1:5000 dilution (*V/V*) of biotin-labeled secondary antibody (Boster, China) at 37℃ for 60 min. The system was hybridized with streptavidin-biotin complex (SABC, Boster, China) at 37℃ for 30 min. DAB stain was performed after rinsed by PBS and followed with hematoxylin stain. After gradient dehydration, transparent, and sealed by neutral gum, the results were observed and photographed by microscopy.

2.2 Isolation and identification of hepatocytes nucleus

Nucleus isolation from hepatocytes is performed with a reported procedure (Matunis, 2006). Working in a 4 ℃ cold room, all buffers are kept on ice at near 0℃, chilled hepatocytes are added to 20 ml of freshly prepared, ice-cold buffer A (0.25 M sucrose, 50 mM Tris–HCl (pH 7.5), 25 mM KCl,5 mM MgCl2, 2 mM DTT, 1×protease inhibitors). The hepatocytes in buffer A are homogenized using a 20 ml capacity Potter-Elvehjem homogenizer, then centrifuged at 800g for 20 min to obtain a crude nuclear pellet. During the centrifugation, 5 ml of buffer B (2.3 M sucrose, 50 mM Tris–HCl (pH 7.5), 25 mM KCl, 5 mM MgCl2, 2 mM DTT, 1×protease inhibitors) is added to the bottom of 6 Beckman SW28 ultracentrifuge tubes. Following centrifugation, the supernatant is removed carefully avoid disturbing the loose nuclear pellet. The nuclear pellet is gently resuspended in ice-cold buffer B and added 30 ml to each of the six SW28 ultracentrifuge tubes, overlaying the 5 ml of previously added buffer B. Samples are centrifuged at 27,000 rpm (141,000g) at 4℃ for 1 h. Following centrifugation, remove and discard the layer of lipid formed at the top of the tubes. The remaining supernatant is poured off by rapidly inverting the tubes, and residual liquid is allowed to drain from the tubes by placing them upside down on paper towels. Gimsa stain was performed to identify the purity of isolated nucleus.

2.3 Sample preparation for 2D gel electrophoresis (2-DE)

The protein of nucleus was homogenized in lysis buffer containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 1% (v/v) DTT, 2% (v/v) immobilized pH gradient buffer (IPG buffer, pH 3–10), and 1% (v/v) protease inhibitor cocktail. The nuclear lysis was confirmed by the addition of Trypan Blue and examination under a microscope. The homogenized mixture was centrifuged at 40,000 × g for 1 h at 4℃, and then the supernatants were collected and stored at -80℃ until further analysis. The protein concentration was measured using the PlusOne 2-D Quant kit (GE Healthcare, USA).

2.4 Two-dimensional gel electrophoresis

2-Dimensional gel electrophoresis (2-DE) was performed as described previously (Huo et al.2008). Isoelectric focusing (IEF) was carried out on an Ettan IPGphor Ⅲ (Amersham Bioscience) with 24-cm immobilized pH gradient strips (pH3~10 NL; Amersham Bioscience). Samples containing 500 μg of protein were mixed with rehydration solution containing 8 M urea, 2% CHAPS, 20 mM DTT, 0.5% (v/v) IPG buffer (pH 3–10), and 0.001% bromphenol blue. Focusing parameters were conducted by stepwise increase of the voltage as follows: 30 V for 12 h, 200 V for 1 h, 500 V for 1 h, 1000 V for 1 h, 3500 V for 1 h, and 8000 V for 12 h. Strips were equilibrated at room temperature for first 15 min in equilibrated solution containing 6 M urea, 50 mM Tris-HCL, 30% (v/v glycerol, 2% (w/v) SDS, and 1% (w/v) DTT, and incubated for another 15 min in similar solution by replacing DTT with 2.5% (w/v) iodoacetamide. The second dimension separation was run on 11% SDS gel in Ettan DALT6 (GE Healthcare, Sweden) with 1 W per gel for first 60 min and followed by 13 W per gel until the bromphenol blue line reached the bottom of the gels.

2.5 Image scanning, analysis, and in-gel digestion

All gels were visualized by Coomassie blue G250 staining and scanned at 600 dpi resolution (ImageScan Ⅲ, Epson, USA). Spots detected by ImageMaster 2D Platinum 6.0 software (Amersham Biosciences).The protein spots were excised from the gel, diced into small pieces of approximately 1 mm^3, washed three times with ACN, dried for 30 min at room temperature and digested according to the reported procedure (Cheng et al., 2005).

2.6 MALDI-TOF MS and PMF

For acquisition of MS peptide maps of the proteins, 1 μl aliquots of the generated cleavage products were dispensed onto the MTP AnchorChip™

800/384 sample support, followed by 1 µl of CHCA matrix solution (solution of a-cyano-4-hydroxycinnamic acid in 35% ACN, 0.1% TFA). Samples were analyzed on a AutoFlex III MALDI-TOF mass spectrometer (Bruker Daltonics, Germany). All spectra were acquired in positive-ion reflector mode. Peptide mass fingerprint (PMF) and MS data were analyzed using the MASCOT search engine version 2.3 (Matrix Science, London, UK).

2.7 Bioinformatics analysis

Sequences of identified proteins were submitted to NCBI BLAST server to find similar sequences. The online software COMPUTE PI/MW (http://web.expasy.org/compute_pi/) tool was used to predict and verify the molecular weight (mw) and isoelectric point (pI). Gene ontology (GO) annotations for identified proteins based on MetaCore and Ingenuity Pathway Analysis（IPA）analysis.

3. Results

3.1 immunochemical identification of hepatocytes

To verify the purity of isolated hepatocytes, we examined the expression of hepatocytes specific markers by cell immunochemical staining. Almost all hepatocytes were positive for ALB antibody (Figure.1). Statistical results showed that about 97% of all cells were just positive for albumin expression.

3.2 Purity of isolated hepatocytes nucleus

The crude nucleus from the separate process and final obtained pure nucleus were examined by Gimsa staining; microscopic statistics showed the finally obtained nucleus get purity above 98%, no intact cell can be seen on the slide (Figure.2).

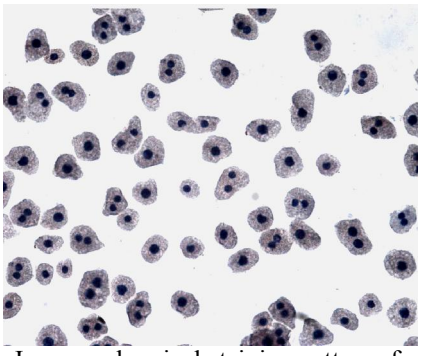

Figure 1. Immunochemical staining patterns for ALB in theisolated hepatocytes. (400X)

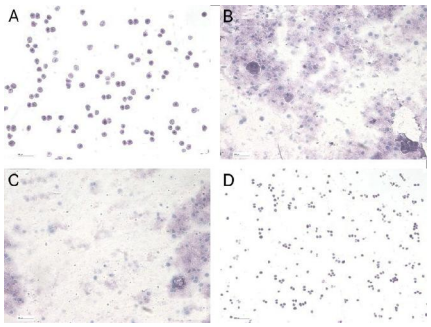

Figure 2. Purity of isolated hepatocytes nucleus (100X)

Gimsa staining of isolated hepatocytes (A), crude nucleus in buffer A after homogenized (B), crude nucleus after centrifuged at 800g (C) and finally obtained pure nucleus (D).

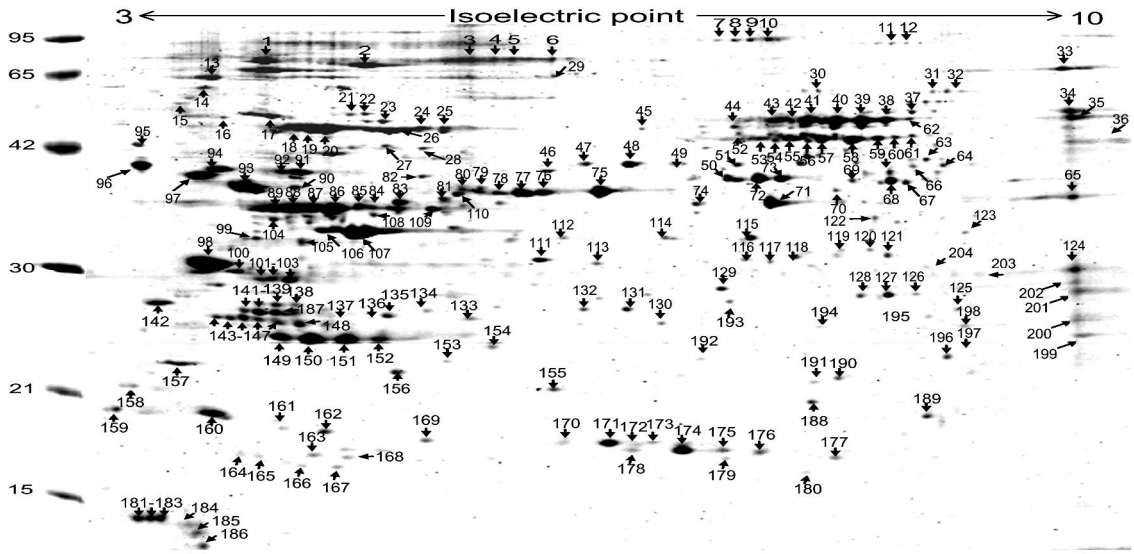

Figure 3. 2-DE profile of hepatocytes nucleoprotein on Coomassie blue-stained gel over the pH ranges 3–10 NL. The numbers in the figure are the spot of 204 kinds of nucleoproteins identified by MALDI-TOF MS

3.3 2-DE map of hepatocytes nucleoprotein and MALDI-TOF MS

The nucleoprotein from hepatocytes nucleus was analyzed by 2-DE to draw a protein profile by ImageScan scanner Ⅲ. The proteins were well represented by 2-DE separations across a gradient of pH 3–10 NL. 2-DE map constructed with Coomassie blue G250-stained gels showed a total of 627 protein spots were detected by ImageMaster 2D Platinum software (Figure.3). Protein gel spots were excised and prepared for MALDI-TOF MS analysis. MALDI–TOF MS identification and database search (http://www.psort.org/) predicted that 204 proteins were subcellular located to nucleus (Table.1).

3.7 Bioinformatics analysis

COMPUTE PI/MW used to predict and verify the molecular weight (mw) and isoelectric point (pI), results were shown in Table1. In order to better understand the biological functions of these nucleoproteins, gene ontology annotation was performed by MetaCore and Ingenuity Pathway Analysis (IPA) analysis. The results showed that the nucleoprotein involved in important physiological activities in rat liver (Figure.4). Ingenuity Pathway Analysis showed that these nucleoproteins were involved in the process of cell cycle regulation, DNA replication, recombination and damage repair, cell assembly and organization, cell development, nervous system development and function, cancer, cardiovascular system development and function, cell death and survival, cell morphology, Cell-To-Cell signaling and interaction, Cell-mediated immune response, Cellular growth and proliferation, and so on. These proteins are classified according to the number of proteins involved in different molecular functions. In the identified nucleoproteins, 22 kinds were involved in the cell cycle, 18 kinds were involved in cell development, and 13 kinds were involved in nervous system development and function, 12 kinds were involved in DNA replication, recombination and damage repair, Details as shown below (Figure.5).

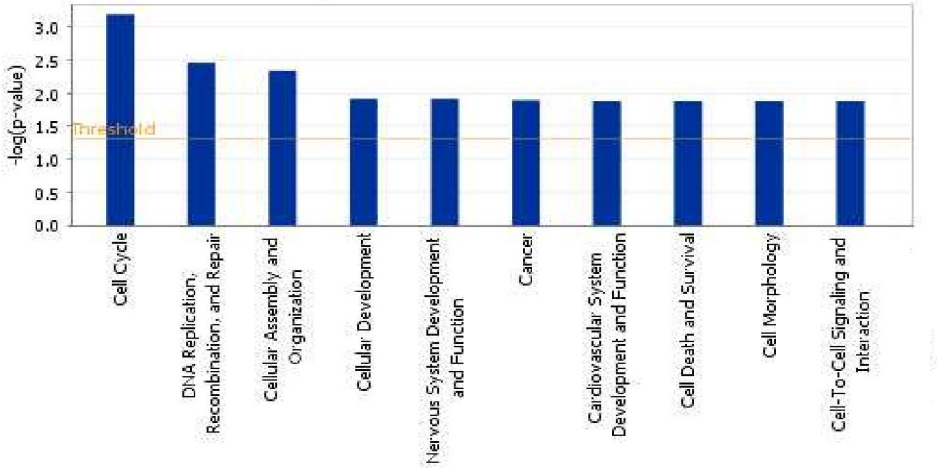

Figure 4. Top 10 of the physiological activities nucleoprotein involved in rat liver.

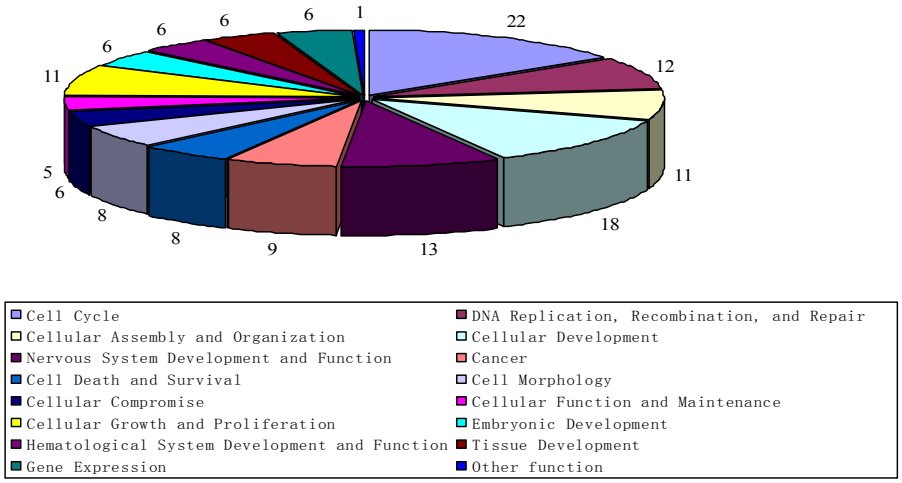

Figure.5 Classification according to the number of proteins involved in different molecular functions.

Table 1 – Proteins Identificated in hepatocyte nucleus of Rat

Spot ID[a)]	Protein Name	Accession no.[b)]	MW(kDa)	pI	Mascot score[c)]	MS Coverage
1	Secernin-1	SCRN1_RAT	46994	4.6	65	21
2	Heat shock cognate 71 kDa protein	HSP7C_RAT	71055	5.2	56	17
3	Exportin-1	XPO1_RAT	124099	5.7	54	4
4	Ankyrin repeat and LEM domain-containing protein 2	ANKL2_RAT	104268	5.7	59	93
5	RNA polymerase II-associated protein 1	RPAP1_RAT	156258	6.2	54	16
6	Mediator of RNA polymerase II transcription subunit 24	MED24_RAT	111606	6.3	54	11
7	PREDICTED: Werner syndrome protein	gi|293354272	157167	6.5	69	16
8	Serine/threonine-protein kinase CST20	STE20_CANAL	132977	6.6	77	28
9	ATP-dependent RNA helicase DDX42	gi|157817897	102290	6.6	64	7
10	Serine/threonine-protein kinase mTOR	MTOR_RAT	290662	6.8	65	6
11	Structural maintenance of chromosomes protein 1A	SMC1A_RAT	143743	8.2	51	13
12	transformation/transcription domain-associated protein	gi|149034906	372069	9.4	50	11
13	Nucleophosmin	NPM_RAT	32711	4.5	65	25
14	Secretoglobin family 1C member 1	SG1C1_RAT	10565	4.2	65	12
15	Growth arrest and DNA damage-inducible protein GADD45 alpha	GA45A_RAT	18810	4.4	92	18
16	Eukaryotic translation initiation factor 6	IF6_RAT	27067	4.5	96	37
17	DNA-directed RNA polymerase II subunit RPB3	gi|29336059	31766	4.6	68	21
18	GATA binding protein 4	GATA4_RAT	1641	7.6	75	6
19	heterogeneous nuclear ribonucleoproteins C1/C2	gi|283436180	32360	4.8	83	11
20	Lamin-B1	LMNB1_RAT	66794	5	52	11
21	Splicing factor 3A subunit 3	SF3A3_HUMAN	59154	5.1	52	9
22	similar to Lmnb2 protein	LMNB2_Rat	56757	5.2	47	12
23	Protein cereblon	CRBN_RAT	51700	5.2	60	6
24	Protein QN1 homolog	QN1_RAT	161131	5.5	54	24
25	Dynactin subunit 1	DCTN1_RAT	142583	5.5	56	15
26	Heterogeneous nuclear ribonucleoprotein F	HNRPF_RAT	46043	5.2	76	14
27	Interferon-induced guanylate-binding protein 2	GBP2_RAT	67637	5.4	64	7
28	ruvB-like 2	gi|6755382	51252	5.4	51	10
29	BRCA1-A complex subunit RAP80	UIMC1_RAT	81585	6.3	82	8
30	Transcription factor 4	ITF2_RAT	63243	7.1	78	25
31	Histone-lysine N-methyltransferase EZH1	EZH1_HUMAN	87325	9.1	89	34
32	B-cell CLL/lymphoma 6	D3ZTN3_RAT	80582	9.5	53	18
33	Large proline-rich protein BAT2	BAT2_RAT	229421	10	91	20
34	Zinc finger RNA-binding protein	ZFR_RAT	118113	9.7	92	13
35	Putative tRNA pseudouridine synthase C25B8.05	YL85_SCHPO	52085	9.6	64	14
36	PREDICTED: vasoactive intestinal peptide receptor 1	VIPR1_RAT	84473	11	68	6
37	Protein kinase C delta type	KPCD_RAT	78724	9	70	13
38	T-box transcription factor TBX3	TBX3_RAT	79684	8.6	61	6
39	Na(+)/H(+) exchange regulatory cofactor NHE-RF2	NHRF2_RAT	37688	8	45	9
40	DNA polymerase delta catalytic subunit	DPOD1_RAT	124948	7.8	61	25
41	TRM1-like protein	TRM1L_RAT	81388	7.4	96	9
42	similar to cofactor required for Sp1 transcriptional activator	D4ABU0_RAT	99680	10	109	26
43	Serine/arginine-rich splicing factor 5	SRSF5_RAT	30987	12	56	23
44	zinc finger protein 580	gi|254540130	19167	12	64	33
45	Crooked neck-like protein 1	CRNL1_RAT	83649	6.5	54	16
46	Stress-70 protein, mitochondrial	GRP75_RAT	74097	5.9	60	7
47	Eukaryotic initiation factor 4A-III	IF4A3_RAT	47096	6.3	67	34
48	Argininosuccinate synthase	ASSY_RAT	46752	8.5	67	9
49	Heterogeneous nuclear ribonucleoprotein A3	ROA3_RAT	39856	9.6	62	14
50	PREDICTED: upstream binding transcription factor	UBF1_RAT	21902	9.5	79	15

Table 1 – Proteins Identificated in hepatocyte nucleus of Rat *(Continued)*

Spot ID[a)]	Protein Name	Accession no.[b)]	MW(kDa)	pI	Mascot score[c)]	MS Coverage
51	Heart- and neural crest derivatives-expressed protein 2	HAND2_RAT	23765	9.8	76	75
52	Zinc finger protein 90	ZFP90_RAT	74046	9.9	62	16
53	40S ribosomal protein S6	RS6_RAT	28834	12	50	8
54	Signal recognition 54 kDa protein	SRP54_THENV	47966	9.7	57	10
55	Arrestin domain-containing protein 4	ARRD4_RAT	33880	9.4	116	19
56	DNA topoisomerase I	TOP1M_RAT	69652	10	68	31
57	Tristetraprolin	TTP_RAT	34260	10	51	6
58	T-box transcription factor TBX1	gi|157823231	51913	9.5	69	9
59	splicing factor, arginine/serine-rich 5	gi|149025039	12748	11	87	3
60	Queuine tRNA-ribosyltransferase	TGT_RAT	44842	8.9	61	93
61	Centromere protein N	CENPN_RAT	39776	9	55	24
62	Cryptochrome-1	CRY1_RAT	66930	9.3	53	11
63	mRNA export factor	RAE1L_RAT	41463	9.1	94	14
64	Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2_RAT	37512	9.3	72	7
65	Replication initiator 1	REPI1_RAT	63907	12	79	21
66	Serine/arginine-rich splicing factor 3	SRSF3_BOVIN	19546	12	98	11
67	Ubiquitin-60S ribosomal protein L40	RL40_RAT	15004	11	75	7
68	Fibronectin type 3 and ankyrin repeat domains 1 protein	FANK1_RAT	38615	8.7	85	31
69	Probable DNA primase small subunit	PRI1_HUMAN	38152	9.5	79	10
70	Synaptonemal complex protein 3	SYCP3_RAT	29828	9.5	61	17
71	TGF-beta-activated kinase 1 and MAP3K7-binding protein 2	TAB2_RAT	76794	9.6	51	11
72	Prokineticin-2	PROK2_RAT	12213	12	50	12
73	LIM domain-containing protein 2	LIMD2_RAT	14626	10	78	14
74	Centromere protein W	CENPW_RAT	10082	12	56	15
75	Splicing factor, proline- and glutamine-rich	SFPQ_MOUSE	75508	9.9	78	29
76	60S ribosomal protein L36	RL36_RAT	12317	12	52	14
77	splicing factor, arginine/serine-rich 1 (ASF/SF2)	gi|148669916	18717	12	63	12
78	similar to yippee-like 3 (predicted), isoform CRA_e	D4A0Y3_RAT	8666	12	51	23
79	splicing factor, arginine/serine-rich 7	gi|119620768	15981	11	62	87
80	similar to ankyrin repeat domain 29	gi|149031746	13018	9.9	61	95
81	Mitogen-activated protein kinase 9	MK09_RAT	48499	5.5	52	81
82	PREDICTED: centrosomal protein 27 isoform 3	gi|293355322	25128	5.5	56	14
83	Steroid receptor RNA activator 1	SRA1_RAT	25533	5.6	58	23
84	mortality factor 4 like 2, isoform CRA_d	MO4L2_RAT	2935	9.9	59	12
85	E3 ubiquitin-protein ligase parkin	PRKN2_RAT	53613	6.5	96	38
86	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase	MTND_RAT	21505	5.1	57	87
87	PRP39 pre-mRNA processing factor 39 homolog	D3ZUB5_RAT	35104	5	110	30
88	Transforming growth factor beta-1-induced transcript 1 protein	TGFI1_RAT	51686	6.5	70	8
89	PREDICTED: Nedd4 binding protein 1-like	gi|293357651	24507	10	57	13
90	NF1-X1	O70189_RAT	18659	8	53	10
91	Complexin-2	CPLX2_RAT	15499	4.9	59	25
92	Homeobox protein Hox-A7	HXA7_RAT	12601	4.7	80	14
93	S-phase kinase-associated protein 1	SKP1_RAT	18831	4.2	51	12
94	Heat shock protein beta-8	HSPB8_RAT	21750	4.8	70	12
95	Calreticulin	CALR_RAT	48137	4.2	91	16
96	Protamine-3	PRM3_RAT	11557	4.1	66	27
97	SOSS complex subunit B1	SOSB1_RAT	22534	9.9	62	16
98	Galectin-7	LEG7_RAT	15333	6.5	61	10
99	XPA binding protein 2, isoform CRA_a	:0CYF8_MOUSE	42530	4.7	51	30
100	D(1A) dopamine receptor	DRD1_RAT	50308	6.5	61	16
101	Phosphatidylinositol-5-phosphate 4-kinase type-2 alpha	PI42A_RAT	46409	6.5	101	12
102	Abl interactor 1	ABI1_RAT	51787	6.6	67	24

Table 1 – Proteins Identificated in hepatocyte nucleus of Rat *(Continued)*

Spot ID[a)]	Protein Name	Accession no.[b)]	MW(kDa)	pI	Mascot score[c)]	MS Coverage
103	Nuclear pore complex protein Nup54	NUP54_RAT	55825	6.6	69	13
104	Histone-binding protein RBBP7	RBBP7_RAT	48132	4.8	51	6
105	Small muscular protein	SMPX_RAT	9115	11	68	21
106	Normal mucosa of esophagus-specific gene 1 protein	NMES1_RAT	9592	11	66	13
107	Histone promoter control protein 2	HPC2_SCHPO	36664	6.4	76	32
108	DNA-directed RNA polymerases I, II, and III subunit RPABC1	RPAB1_RAT	24669	5.6	69	15
109	Prohibitin	PHB_RAT	29859	5.5	50	12
110	Nup37 protein	gi|171846615	33606	5.7	69	15
111	GTP-binding nuclear protein Ran	RAN_RAT	24579	7.8	71	21
114	dual specificity protein phosphatase 15	gi|157820053	15130	12	99	32
115	BET1-like protein	BET1L_RAT	12466	9.7	59	7
116	ribosomal RNA-processing protein 7 homolog A	gi|194474082	32492	10	52	82
117	Fatty acid-binding protein, adipocyte	FABP4_RAT	14813	9	87	19
118	Prolactin regulatory element-binding protein	PREB_RAT	45899	9.8	67	8
119	Zinc finger protein SNAI2	SNAI2_RAT	30772	10	74	17
120	Ribosomal RNA-processing protein 8	RRP8_SCHPO	36591	10	64	18
121	Uncharacterized protein C1orf103 homolog	LRIF1_RAT	83074	10	64	24
122	endonuclease/reverse transcriptase	Q4JFW1_RAT	150811	10	58	24
123	Small nuclear ribonucleoprotein-associated protein B	RSMB_RAT	21751	12	59	46
124	Ubiquitin carboxyl-terminal hydrolase 2	UBP2_RAT	70118	10	50	12
125	Homeobox protein Hox-D3	HXD3_RAT	13207	12	59	5
126	Pre-mRNA-splicing factor SYF2	SYF2_RAT	28752	9.2	67	10
127	Transcription termination factor	MTERF_RAT	43366	10	65	6
128	CDK105	NSA2_RAT	26157	11	44	7
129	Homeobox protein Hox-C4	HXC4_RAT	9401	11	70	28
130	histone H1	H11_RAT	1114	9.7	51	31
131	Insulin gene enhancer protein ISL-1	ISL1_RAT	39923	9.8	88	13
132	patatin	PLPL7_MOUSE	2126	9.9	58	6
133	Protein mago nashi homolog	MGN_RAT	17210	5.7	61	10
134	Spindle and kinetochore-associated protein 2	SKA2_RAT	16678	5.6	56	88
135	Transcription factor A	TFAM_RAT	28397	10	51	11
136	synaptonemal complex protein 1	SYCP1_RAT	3969	10	177	16
137	ADP-ribosylation factor-like protein 4A	ARL4A_RAT	22745	10	59	22
138	PREDICTED: similar to Tripartite motif protein 30-like	TR30A_MOUSE	21361	6.4	89	12
139	core-binding factor subunit beta	gi|61557239	21732	6.4	109	15
140	Guanine nucleotide exchange factor MSS4	MSS4_RAT	14261	5	72	13
140	cyclin C, isoform CRA_c	CCNC_RAT	23229	6.4	76	16
141	Protein S100-A4	S10A4_RAT	11997	4.9	124	15
142	Oncomodulin	ONCO_RAT	12238	3.9	80	19
143	Myosin regulatory light chain 12B	ML12B_RAT	19883	4.6	52	36
144	60S acidic ribosomal protein P2	RLA2_RAT	11685	4.2	64	22
145	cAMP-dependent protein kinase inhibitor alpha	IPKA_RAT	7956	4.3	52	41
146	DNA-directed RNA polymerase II subunit RPB7	RPB7_RAT	19453	5.2	59	10
147	General transcription factor IIH subunit 2	TF2H2_RAT	45758	6.6	81	8
148	Prelamin-A/C	LMNA_RAT	74564	6.6	84	19
149	breast carcinoma amplified sequence 2	B5DFM8_RAT	24131	5	91	19
150	Keratin, type II cytoskeletal 8	K2C8_RAT	53985	5.7	93	21
151	Protein S100-A5	S10A5_RAT	10862	5	71	16
152	Small ubiquitin-related modifier 2	SUMO2_RAT	10921	5.2	62	13
153	Barrier-to-autointegration factor	BAF_RAT	10266	5.7	60	57
154	Ester hydrolase C11orf54 homolog	CK054_RAT	35427	6.2	64	26
155	Secretoglobin family 2A member 1	SG2A1_RAT	10900	6.3	52	28
156	Barrier-to-autointegration factor-like protein	BAFL_MOUSE	10659	5.2	53	12
157	Prothymosin alpha	PTMA_RAT	12375	3.6	72	18
158	Acidic leucine-rich nuclear phosphoprotein 32 family member	AN32A_RAT	28718	3.8	55	26
159	RNA polymerase beta subunit	gi|2258104	1404	3.9	69	17

Table 1 – Proteins Identificated in hepatocyte nucleus of Rat *(Continued)*

Spot ID[a)]	Protein Name	Accession no.[b)]	MW(kDa)	pI	Mascot score[c)]	MS Coverage
160	Protein S100-A3	S10A3_RAT	12253	4.6	52	19
161	THO complex subunit 3	gi|157823413	39455	5.7	96	15
162	Histone H4	H4_RAT	11360	12	56	33
163	Ubiquitin-fold modifier 1	UFM1_RAT	9169	10	53	25
164	Thioredoxin	THIO_RAT	12008	4.6	110	39
165	beta-A3 crystallin	gi|2338452	6053	4.9	54	15
166	rCG34610, isoform CRA_a	D4A9L2_RAT	24679	11	58	34
167	FUS interacting protein (serine-arginine rich) 1	Q4KM38_RAT	20016	11	59	6
168	Homeobox protein Hox-C5	HXC5_XENLA	11108	11	67	30
169	Calcium/calmodulin-dependent protein kinase II inhibitor 2	CK2N2_XENLA	8711	5.7	60	12
170	sestrin-3	gi|157822219	57292	5.8	134	12
171	26S protease regulatory subunit 4	PRS4_RAT	49325	5.8	65	13
172	UPF0729 protein C18orf32 homolog	CR032_RAT	8334	10	77	15
173	Ubiquitin fusion degradation protein 1 homolog	UFD1_RAT	34748	6.3	72	17
173	Homeobox protein Hox-B8 (Fragment)	HXB8_RAT	11408	11	60	23
174	dihydrofolate reductase (DHFR)	DYR_RAT	1550	7	56	7
175	Enhancer of yellow 2 transcription factor	ENY2_DROMO	10798	6.8	64	21
176	elongation factor 1 alpha	gi|13359291	1891	7	74	8
177	p27kip1	gi|4587316	962	11	97	33
178	similar to RIKEN cDNA 1110031B06	gi|149052798	962	6.9	55	23
179	40S ribosomal protein S27	RS27_RAT	9797	11	57	12
180	probasin	PBAS_RAT	3059	11	54	13
181	CDKN2	gi|1483509	1992	3	89	17
182	mouse double minute 2 protein	MDM2_MOUSE	2311	3.2	73	21
183	Kruppel-like factor 3	gi|57833895	2033	3.7	83	38
184	Serine/threonine-protein phosphatase PP1-beta catalytic subunit	PP1B_RAT	37961	5.8	73	9
185	Serpin B10	SPB10_RAT	45415	6.1	58	19
186	histidine triad nucleotide binding protein W	APTX_RAT	2448	4.7	65	26
187	Pre-mRNA-processing factor 19	PRP19_RAT	55661	6.1	63	11
188	Anaphase-promoting complex subunit CDC26	CDC26_RAT	9796	7.2	54	13
189	Spermatid nuclear transition protein 1	STP1_RAT	6392	13	89	10
190	Arrestin-D (Fragment)	ARRD_RAT	7921	7.9	65	82
191	PEST proteolytic signal-containing nuclear protein	PCNP_PONAB	18955	7.7	86	8
192	histone H1.a, hepatic	gi|2118968	5377	12	69	5
193	40S ribosomal protein S28	RS28_RAT	7893	12	57	26
194	oligodendrocyte transcription factor 2	gi|90959767	2894	9.9	55	12
195	SNRPN upstream reading frame protein	SNURF_RAT	8487	12	79	8
196	Enhancer of mRNA-decapping protein 1	EDC1_YEAST	19283	12	85	17
197	Histone H2B type 1	H2B1_RAT	13982	11	67	9
198	serine/arginine-rich splicing factor 1 isoform 1	gi|5902076	27842	11	65	6
199	Serine/arginine-rich splicing factor 7	SRSF7_BOVIN	27153	13	68	13
200	Serine/arginine-rich splicing factor 2	SRSF2_RAT	25461	12	144	18
201	A/G-specific adenine DNA glycosylase	MUTYH_RAT	58567	11	50	15
202	Heterochromatin protein 1-binding protein 3	HP1B3_RAT	61054	10	69	6
203	probable rRNA-processing protein EBP2	gi|56790289	34837	11	76	14
204	Ubiquitin-conjugating enzyme E2 S	UBE2S_RAT	24371	9.3	61	17

a) Spot ID is the unique number from fig3.
b) Accession no. is the MASCOT result of MALDI-TOF/TOF searched from the Uniprot or NCBI database.
c) A score more than 53 is recognized to be significant ($p<0.05$).

4. Discussion

As the largest, highly structured and most important organelles of eukaryotic cells, Nuclei contain the genetic information that defines the appearance and behavior of an organism. DNA replication, RNA transcription and transcription products processing all are performed in the nucleus (Dundr and Misteli, 2001). So, nucleus is considered to be the control center of the genetic and metabolic in the cell. Therefore, the study of nuclear protein helps to further understand the structure and function of the nucleus. This article combined the subcellular grade and proteomics technology to present for the first time an experimentally determined core nuclear proteome for rat hepatocyte. Our analysis of the nucleus proteome from hepatocyte led to identification of more than 200 proteins reflects the structural and functional complexity of liver nucleus. Besides a large number of

proteins implicated in known nuclear processes, lots of detected proteins carry no functional annotation. Further, extensive bioinformatics approaches using Ingenuity Pathway Analysis revealed the function categories of these nucleoproteins. Importantly, our analysis identified these nucleoproteins implicated in lots of signal transduction pathways, and sheds important new light on nuclear compartments and functions.

Our study provides comprehensive new insight into the biology of the rat hepatocyte nucleus and will serve as an important platform for revealing the expression varies of nucleus protein under certain physiological or pathological conditions. Moreover, it will provide an important resource for searching the valuable marker of diseases diagnosis and therapeutic. And will be of great benefit to future studies that can lead to a better understanding of the process and molecular mechanism of liver disease.

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References

1. Aggarwal K, Lee KH. Functional genomics and proteomics as a foundation for systems biology. Brief Funct Genomic Proteomic. 2003, 2(3):175-184.
2. Cheng GF, Lin JJ, Feng XG, Fu ZQ, Jin YM, Yuan CX, Zhou YC, Cai YM. Proteomic analysis of differentially expressed proteins between the male and female worm of Schistosoma japonicum after pairing. Proteomics. 2005, 5(2):511-521.
3. Duclos S, Desjardins M. Organelle proteomics. Methods Mol Biol. 2011; 753:117-128.
4. Dundr M, Misteli T. Functional architecture in the cell nucleus. Biochem J. 2001, 356:297-310.
5. Fernando H, Wiktorowicz JE, Soman KV, Kaphalia BS, Khan MF, Shakeel Ansari GA. Liver proteomics in progressive alcoholic steatosis. Toxicol Appl Pharmacol. 2013, 266(3):470-480.
6. Huo R, He Y, Zhao C, Guo XJ, Lin M, Sha JH. Identification of human spermatogenesis-related proteins by comparative proteomic analysis: a preliminary study. Fertil Steril. 2008, 90 (4):1109–1118.
7. Jung E, Hoogland C, Chiappe D, Sanchez JC, Hochstrasser DF.. The establishment of a human liver nuclei two-dimensional electrophoresis reference map. Electrophoresis. 2000, 21(16):3483-3487.
8. Lamond AI, Earnshaw WC. Structure and function in the nucleus. Science. 1998, 280:547-553.
9. Lewis JD, Tollervey D. Like attracts like: getting RNA processing together in the nucleus. Science. 2000, 288:1385-1389.
10. Matunis MJ. Isolation and fractionation of rat liver nuclear envelopes and nuclear pore complexes. Methods. 2006 , 39(4):277-283.
11. Misteli T, Spector DL. The cellular organization of gene expression. Curr Opin Cell Biol. 1998, 10:323-331.
12. Mulvey CM, Tudzarova S, Crawford M, Williams GH, Stoeber K, Godovac-Zimmermann J. Subcellular proteomics reveals a role for nucleo-cytoplasmic trafficking at the DNA Replication Origin Activation Checkpoint. J Proteome Res. 2013, DOI: 10.1021/pr3010919.
13. Pandey A, Mann M. Proteomics to study genes and genomes. Nature. 2000, 405(6788):837-846.
14. Patterson SD, Aebersold RH. Proteomics: the first decade and beyond. Nat Genet. 2003, 33:311-3234.
15. Paulo JA, Gaun A, Kadiyala V, Ghoulidi A, Banks PA., Conwell DL, Steen H. Subcellular Fractionation Enhances Proteome Coverage of Pancreatic Duct Cells. BBA-Proteins Proteom. 2013, DOI: 10.1016/j.bbapap.2013.01.011.
16. Phair RD, Misteli T. High mobility of proteins in the mammalian cell nucleus. Nature. 2000, 404(6778):604-609.
17. Qazi AS, Sun M, Huang Y, Wei Y, Tang J. Subcellular proteomics: Determination of specific location and expression levels of lymphatic metastasis associated proteins in hepatocellular carcinoma by subcellular fractionation. Biomed Pharmacother. 2011, 65(6):407-416.
18. Schmid MB. Structural proteomics: the potential of high-throughput structure determination. Trends Microbiol. 2002, 10(10supp1):S27-S31.
19. Shakib K, Norman JT, Fine LG, Brown LR, Godovac-Zimmermann J. Proteomics profiling of nuclear proteins for kidney fibroblasts suggests hypoxia, meiosis, and cancer may meet in the nucleus. Proteomics. 2005, 5(11):2819-2838.
20. Tung CL, Lin ST, Chou HC, Chen YW, Lin HC, Tung CL, Huang KJ, Chen YJ, Lee YR, Chan HL. Proteomics-based identification of plasma biomarkers in oral squamous cell carcinoma. J Pharm Biomed Anal. 2013, 75:7-17.
21. Vondran FW, Katenz E, Schwartlander R, Morgul MH, Raschzok N, Gong X, Cheng X, Kehr D, Sauer IM. Isolation of primary human hepatocytes after partial hepatectomy: criteria for identification of the most promising liver specimen. Artif Organs. 2008, 32(3):205-213.

3/3/2013