References

[1] Blom AB, van der Kraan PM, van den Berg WB. Cytokine targeting in osteoarthritis. Curr Drug Targets. 2007;8(2):283-92.

[2] Ruiz-Romero C, Lopez-Armada MJ, Blanco FJ. Proteomic characterization of human normal articular chondrocytes: a novel tool for the study of osteoarthritis and other rheumatic diseases. Proteomics. 2005;5(12):3048-59.

[3] Ruiz-Romero C, Carreira V, Rego I, Remeseiro S, Lopez-Armada MJ, Blanco FJ. Proteomic analysis of human osteoarthritic chondrocytes reveals protein changes in stress and glycolysis. Proteomics. 2008;8(3):495-507.

Identification of differential proteins in liver cells upon depletion of prohibitin

Virginia Sánchez-Quiles, Enrique Santamaría, Laura Sesma, Fernando J. Corrales

Division of Hepatology and Gene Therapy, Proteomics Unit. Centre for Applied Medical Research (CIMA), University of Navarra. 31008, Pamplona, Spain

Introduction

Prohibitin (Phb) is a multifunctional protein participating in a plethora of essential cellular functions, such as cell signalling, apoptosis, survival and proliferation, playing a central role in the maintenance of liver homeostasis through the regulation of central proteins involved in these processes by means of protein-protein interaction mechanisms. In the liver, deficient prohibitin activity participates in the progression of non-alcoholic steatohepatitis (NASH) and obesity, according to mechanisms that still must be elucidated. Phb1 plays an essential role in hepatic functions such as inhibition of cell proliferation [1, 2] and response to anti-cancer [3] or carcinogenic agents [4]. Down-regulation of Phb1 is an early event in the development of NASH and hepatocellular carcinoma (HCC) in experimental mouse models and humans [5]. All these data suggest that Phb1 may play a prominent role in the progression of HCC.

Materials and Methods

Phb1 expression was silenced in the human HCC cell line PLC/PRF5 using specific siRNAs (siGL, control cells; siPHB, treated cells). Phb1 levels were analysed by immunodetection assay. Apoptosis was quantified with the Cell Death Detection Kit (Roche). Cell proliferation was estimated by cell counting and with the Cell Proliferation Reagent WST-1 (Roche). In order to explore the molecular mechanisms involved in the response of PLC cells to impaired Phb1 activity we used a combination of DIGE and mass spectrometry analyses. To increase the efficiency of the process, cytosolic and microsomal subcellular fractions were isolated (Qproteome Cell Compartment Kit, from Qiagen). Enriched cytosolic and microsomal fractions from siGL and siPHB cells were compared by DIGE analysis. Differential spots detected and quantified by DeCyder were identified by nanoLC-ESI-MS/MS (Q-TOF).

Results

Phb1 levels were reduced by 80% upon siRNA silencing. Apoptosis was quantified and absorbance was 3-fold increased in siPHB cells indicating the proapoptotic effect of Phb deficiency (Figure 1D). In addition, Phb silencing severely compromised the capacity of PLC cells to proliferate in a semisolid substrate, an inherent property of transformed cells that is related with their metastatic capacity (Figure 1C). Both cell counting and formazan production revealed a significant reduction of the proliferation rate of siPHB cells when compared with the control siGL cells (Figure 1A and B).

DeCyder analysis of DIGE of cytosolic and microsomal fractions found 76 and 25 spots diffe-

rentially represented (T test < 0.05) in siPHB with respect to siGL cells. MS/MS analysis allowed the identification of 24 and 6 unique proteins in the cytosolic and microsomal fractions respectively (Table 1).

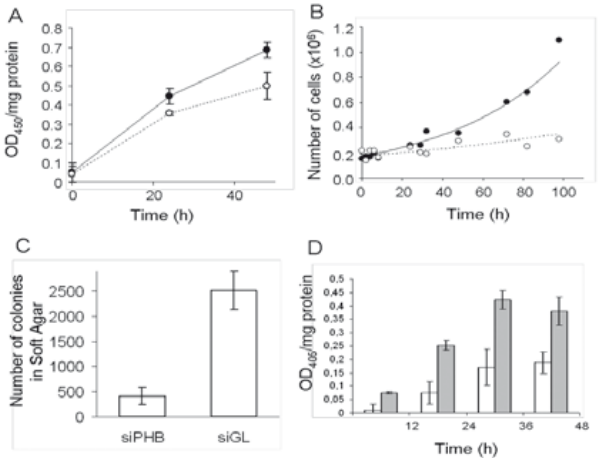

Figure 1. *Cell growth and survival upon Phb1 silencing.*

Conclusions

Phb1 silencing in human hepatoma cell line PLC/PRF5 leads to an increase of apoptotic rate and an impaired cell proliferation. Down-regulation of Calreticulin, ERp29 and Erlin2 (further confirmed by Western blot, figure 2B) in siPHB cells suggest ER stress that, in turn, might participate in the apoptotic response of PLC cells to Phb1 silencing. In agreement to this hypothesis, we found increased CHOP levels, PARP cleavage and activation of caspase 12 and downstream caspase 7 (Figure 2A). ER stress might result from proteasome malfunction leading to the accumulation of miss-folded polypeptide chains in the cell. Phb1 silencing induced down-regulation of proteasome activator complex subunit 2 and stathmin (as verified by Western blot, Figure 2B) suggesting impairment of proteasome activity. Deficient proteasome activity was evidenced by the accumulation of ubiquitinated proteins upon Phb silencing (Figure 2B).

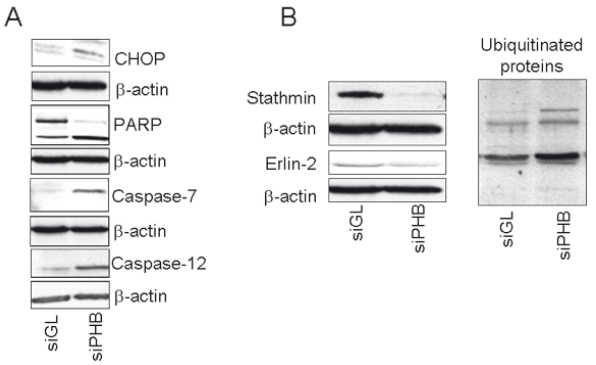

Figure 2. *Molecular targets mediating the cellular response to Phb1 deficiency.*

Table 1. *Identification of proteins corresponding to the differential spots assigned by siGL versus siPHB 2DE gel image analysis.*

Spot	Protein name	Acc num	Mw	pI	Av ratio	T-test	MS Score PLGS	MS Score Phenyx	nº peptides
	Citosolic proteins								
2852	Stathmin	P16949	17291	5,7	-2,01	0,0011	10,9	20,63	3
2122	Thiosulfate sulfurtransferase	Q16762	33407	6,9	1,2	0,044	11,8	12,47	1
2404	Endoplasmic reticulum protein ERp29	P30040	28975	7,3	-1,35	0,029	12,5	55,64	8
2435	Ribosyldihydronicotinamide dehydrogenase	P16083	25935	5,8	-1,45	0,039	1,8	8,58	1
2392	Phosphoserine phosphatase	P78330	24991	5,4	1,55	0,025	7,1	10,78	2
1877	Aspartate aminotrasferase (transaminaseA)	P17174	46218	6,6	1,28	0,048	12,5	17,51	2
1549	Heterogeneous nuclear ribonucleoproteinK	P61978	50944	5,2	-1,2	0,021		11,95	2
1540	Retinal dehydrogenase 1	P00352	54826	6,3	-1,53	0,028	12,5	59,17	7
3017	40S ribosomal protein S12	P25398	14516	6,3	1,2	0,047	2,8	15,74	2
3030	Sulfotransferase1 C2	O00338	34880	7,1	-1,37	0,014		6,35	1
2911	Small ubiquitin-related modifier 3	P55854	11629	5,2	-1,67	0,02	9,3	11,73	1
2782	Protein canopy homolog 2	Q9Y2B0	20639	4,6	-1,34	0,0061		6,68	1
2311	Proteasome activator complex subunit 2	Q9UL46	27344	5,3	-1,36	0,048	11,1	10,68	1
2212	Microtubule-associated protein RP/EB family member 1	Q15691	29980	4,1	-1,33	0,04	10,4	30,4	3
1428	Nucleosome assembly protein 1-like 1	P55209	45345	4,2	-1,71	0,034	11,1	15,43	2
1933	Nucleophosmin	P06748	32554	4,4	1,69	0,014	4	7,94	1
2195	Insulin-like growth factor-binding protein 1	P08833	27885	4,9	-1,91	0,0014	0,8		1
2190	Annexin A4	P09525	35860	5,7	1,2	0,042	12,5	115,29	13
1926	Eukaryotic translation initiation factor 3	Q13347	36478	5,3	-1,44	0,0096	11,4	7,86	1
1799	Creatin kinase B-type	P12277	42617	5,2	-1,56	0,023	12,5	115,93	11
1881	Mitogen-activated protein kinase 1	Q9Y617	40396	7,6	1,35	0,02	7,5	7,32	4
1237	Far upstream element-binding protein 1	Q96AE4	67518	7,4	1,2	0,014	11,8	12,8	2
1674	Rab GDP dissociation inhibitor beta	P50395	50663	6,1	1,21	0,0058		6,07	1
872	Neutral alpha-glucosidase AB	Q14697	106873	5,7	-1,36	0,003	11,4	27,37	4
	Microsomal proteins								
2180	Apolipoprotein A1	P02647	28078	5,3	-1,48	0,0084		6,49	1
1997	Prohibitin	P35232	29785	5,4	-5,44	5,50E-05	11,8	40,59	6
2065	14-3-3 protein epsilon	P62258	29155	4,4	-1,49	0,015	9,4	14,32	2
1582	Erlin 2	O94905	37815	5,3	-1,47	0,002	11,8	31,77	4
1521	Serum paraoxonase/arylesterase 2	Q15165	39373	5,1	-1,56	0,0047	12,5	17,52	2
1528	Creatin kinase B-type	P12277	42617	5,2	-1,6	0,031	10,9	32,62	4

References

[1] McClung JK, Danner DB, Stewart DA, Smith JR, Schneider EL, Lumpkin CK, et al. Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver. *Biochem Biophys Res Commun* 1989; 164: 1316-1322.

[2] Sun Q, Miao M, Jia X, Guo W, Wang L, Yao Z, *et al*. Subproteomic analysis of the mitochondrial proteins in rats 24 h after partial hepatectomy. *J Cell Biochem* 2008; 105: 176-184.

[3] Yoo DR, Jang YH, Jeon YK, Kim JY, Jeon W, Choi YJ, *et al*. Proteomic identification of anti-cancer proteins in luteolin-treated human hepatoma Huh-7 cells. *Cancer Lett* 2009; 282: 48-54.

[4] Leonard JF, Courcol M, Mariet C, Charbonnier A, Boitier E, Duchesne M, *et al*. Proteomic characterization of the effects of clofibrate on protein expression in rat liver. *Proteomics* 2006; 6: 1915-1933.

[5] Santamaria E, Avila MA, Latasa MU, Rubio A, Martin-Duce A, Lu SC, *et al*. Functional proteomics of nonalcoholic steatohepatitis: mitochondrial proteins as targets of S-adenosylmethionine. *Proc Natl Acad Sci U S A* 2003; 100: 3065-3070.

Introduction to Lucid Proteomics System, a new solution for peptide and protein profiling and identification combining Retentate Chromatography to MALDI-TOF and TOF/TOF Mass Spectrometry

Francesco Tortorella

Lucid Sales Manager Europe, Bio-Rad Laboratories

Bio-Rad Laboratories

The Lucid Proteomics System provides a novel means for top-down proteomic analysis of complex biological samples by combining the separation power of SELDI ProteinChip arrays with high-resolution MALDI-TOF and MALDI-TOF/TOF mass spectrometry.

The specificity of the ProteinChip array surfaces permits sample cleanup directly on the arrays, making the process compatible with crude biological samples (such as neat urine) and harsh elution buffer components (salts, urea, etc.) that are not normally compatible with MS analysis.

Several examples of purification strategies and identifications will be described.