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REGULAR ARTICLE

Partial hepatectomy induced liver proteome changes in mice

Christoph W. Strey, Mike S. Winters, Maciej M. Markiewski and John D. Lambris

Department of Pathology & Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA

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Acceleration of liver regeneration could be of great clinical benefit in various liver-associated diseases. However, at present little is known about therapeutic interventions to enhance this regenerative process. Our limited understanding and the complexity of the mechanisms involved have prevented the identification of new targets for treatment. Here we propose a broad-range proteomic approach to this problem that makes possible the simultaneous study of different signaling and metabolic pathways on the liver proteome. Changes in protein expression in mouse livers ($n = 5$ *per* group) at 6 h and 12 h after partial hepatectomy and sham operation, as compared to untreated controls, were analyzed using two-dimensional gel electrophoresis, mass spectrometry (MS), and mass fingerprinting. Twelve proteins, identified by MS, were up-regulated by at least 2-fold after partial hepatectomy. These included adipose differentiation-related protein, gamma-actin, enoyl coenzyme A hydratase 1, serum amyloid A and eukaryotic translation initiation factor 3. These results indicate that liver regeneration following partial hepatectomy affects various signaling and metabolic pathways.

Keywords:
Liver proteomics / Mass spectrometry / Partial hepatectomy

1 Introduction

Liver regeneration continues to be an important focus in mammalian liver research. Previous studies have identified many pathways involved in this process and have charted the time course of major regulating events that control the replication of hepatocytes and enable this large parenchymal organ to restore its functional integrity after pronounced damage or volume loss [1–3]. In this context, partial hepatectomy (PHx) is a classic experimental model of rapid liver cell proliferation [4]. However, replication is not the only challenge confronting the remaining hepatocytes after liver damage. At the same time they have to compensate for increasing metabolic demand and for the requirement to sustain or increase the levels of vital serum proteins in the context of an acute-phase response [5, 6]. This balance between replication, metabolism, and synthesis ensures the survival of the affected organism and, after liver damage, the subsequent restoration of liver function. It is only poorly understood how the liver is able to maintain this balance. Furthermore, it is unknown how these three tasks of cellular function are prioritized, especially under pathophysiological circumstances in which the capacity of the hepatocytes to compensate for parenchymal loss can be impaired or associated with prolonged liver failure.

It would be of great clinical interest to develop therapeutic options to enhance liver regeneration or to support the liver in its attempt to restore its functional integrity under pathophysiological circumstances. However, the complexity of the regulatory mechanisms of liver regeneration and our limited understanding of the functional priorities of the hepatocytes have rendered the identification of targets for therapeutic interventions very difficult. Although many regulatory events and their temporal distribution during liver

Correspondence: Dr. John D. Lambris, Protein Chemistry Laboratory, 401 Stellar-Chance Labs, 422 Curie Blvd., School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
E-mail: lambris@mail.med.upenn.edu
Fax: +1-215-573-8738

Abbreviations: ADRP, adipose differentiation related protein; **DDT**, *D*-dopachrome tautomerase; **ECoAH-1**, enoyl coenzyme A hydratase 1; **MD**, malate dehydrogenase; **PHx**, partial hepatectomy; **SBP2**, selenium binding protein 2; **TIF3**, translation initiation factor 3

regeneration [1–3] have been identified to date, the study of single pathways and signaling factors has thus far been insufficient to fully delineate the metabolic complexity of the proliferative response after liver injury. Several attempts have been undertaken to obtain a more global picture of the cellular processes that occur during liver regeneration. Recently, gene array approaches including up to 6000 genes have been used to map the complex dynamic response of the liver's metabolic network and to allow a pattern analysis of the genes involved [7–10]. Various differentially regulated clusters of genes have been described during the process of liver regeneration. However, approaches that involve studying gene regulation on a post-translational level run the risk of missing out on the effect of post-transcriptional regulatory mechanisms, which alter the abundance and function of the corresponding gene-products [11, 12]. In this context, Mitchell *et al.* [13] have recently underlined the necessity for a proteomic approach to match the microarray data with the post-transcriptional situation of the regenerating liver.

The proteomic approach used in the present study circumvents the pitfalls mentioned above and focuses directly on individual gene products and their isoforms, offering the opportunity to analyze a large number of proteins simultaneously. Several authors have described proteome changes during liver regeneration [14–17]; however, technical limitations at the time made comprehensive liver proteome analysis difficult. In the current study we applied 2-DE in connection with MS [18] and computer database searches to identify differentially regulated proteins during liver regeneration at various time points after PHx in the mouse. Here we describe 12 proteins, identified by MS, that were upregulated by at least 2-fold after PHx. These results indicate that hepatic adaptations to liver regeneration after PHx affect various signaling pathways, the acute-phase response and lipid metabolism.

2 Materials and methods

2.1 Animals

Specific pathogen-free C57BL6 mice, 14 to 18 weeks old, (Jackson Laboratory, Bar Harbor, ME, USA) were used in all experiments. Tissues from various organs, including the resected portion of the liver at the time of PHx, were assessed by histology. None of these tissue samples showed morphologic features of pathological processes. Livers were harvested from five groups of mice ($n = 5$ *per* group): from a reference group that did not undergo surgery (no surgery group) and from mice at 6 or 12 h after either PHx or sham surgery. All animal studies were conducted in compliance with the guidelines of the University of Pennsylvania, according to an animal protocol approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

2.2 PHx

PHx (~70%) was performed according to the method of Higgins *et al.* [4]. The median and left lateral lobes were removed without injuring the remaining liver tissue as described previously [19]. For the sham surgery, a laparotomy was performed and a piece of suture was placed into the abdomen without removal of any liver tissue. All animals were perfused with physiological saline solution before organ harvest. Histological analysis in tissues from internal organs of all animals was performed to exclude pre-existing pathology; any animal showing signs of pathology (*i.e.* infection) were excluded from further analysis.

2.3 2-DE

Pooled livers from five mice in each group (harvested without surgery or at 6 or 12 h after PHx, or 6 or 12 h after sham surgery) were used for 2-DE essentially as described [20]. Livers, frozen at −70°C, were homogenized with a mortar and pestle in liquid nitrogen. The tissue powder was resuspended in lysis buffer consisting of 40 mM Tris, 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM 1,4-dithioerythritol, 1 mM EDTA, and protease inhibitors. The suspension was homogenized by sonication for 30 s and centrifuged at 150 000 × *g* for 1 h. Protein content was determined by the Coomassie blue method [21]. For the first dimension, 2 mg of protein was applied to 24 cm immobilized pH 5–8 linear gradient strips (Bio-Rad, Hercules, CA, USA) with ampholytes (p*I*: 3.5–10; Amersham Biosciences, Piscataway, NJ, USA). IEF using the Proten IEF cell-apparatus (Bio-Rad) was started at 200 V and the voltage was gradually increased to 10 000 V and kept constant until 70 000 Vh were reached. Separation in the second dimension was performed on 12.5% Tris/HCl gels (Amersham, Biosciences). The gels were run at 40 mA *per* gel in a Criterion gel apparatus (Bio-Rad). After protein fixation for 12 h in 40% ethanol containing 10% acetic acid, the gels were stained with Coomassie blue (Gelcode, Pierce, Rockford, IL, USA) for 24 h. After destaining with water for 12 h, the gels were scanned in a densitometer (Molecular Dynamics, Sunnyvale, CA, USA). The resulting digital images were analyzed and quantified using the ImageMaster 2D Elite software (Amersham Biosciences). 2-DE runs were repeated four times for each pooled sample. For comparisons of protein levels among gels, normalization was done against the total intensity of all spots present in the gel. Concentrations of each protein for the four PHx and sham surgery groups were compared to those for the reference group that did not undergo surgery. For the determination of significance ($p < 0.05$) for protein abundance changes between the groups and time points the Mann-Whitney test was applied.

2.4 MALDI-MS analysis and bioinformatics

For MS, the spots of interest were excised from the gels and sequentially washed for 1 h in 50 mM NH_4HCO_3 and in 50% ACN before gel digestion. After washing, gel pieces were

shrunk in ACN, dried and then rehydrated with 50 mM NH_4HCO_3 containing 0.01% sequence-grade trypsin (Promega, Madison, WI, USA). After overnight digestion at room temperature, the peptides were extracted twice with 50% ACN and 0.1% TFA, applying one round of vortexing and sonication (20 min each). After being dried, the peptide mixtures were resuspended in 5 μL 0.1% TFA, mixed 1:1 with CHCA matrix (10 mg/mL in 30% ACN/0.1% TFA, v/v) and spotted on a MALDI plate. MS analysis was performed with a 2E Tofspec laser desorption TOF mass spectrometer equipped with a nitrogen laser operating at 337 nm (Micromass, Manchester, UK). Each mass spectrum was calculated from an average of 80–100 acquisitions (four shots *per* acquisition). MALDI-TOF spectra were calibrated externally using angiotensin I (Sigma, St. Louis, MO, USA and adrenocorticotropic hormone (Sigma). The *m/z* values in each mass spectrum were matched with the corresponding amino acid sequence in the protein using profound (http://129.85.19.192/ profound_bin/ WebProFound.exe). The mass tolerance filter for the monoisotopic precursor ion was set at 0.4 Da and the mass range filter was set according to the M_r and p*I* coordinates of the digested spot on the 2-D gel map.

3 Results

2-DE and Coomassie blue staining allowed for the simultaneous digital quantification of approximately 900–1000 spots in each gel. A total of 29 spots in the gels after PHx displayed a significant and at least 2-fold change in abundance when compared to the reference (no surgery) or sham group (Fig. 1). Seventeen of the corresponding proteins have thus far been unequivocally identified. The identified proteins were classified according to their functional properties and with respect to their kinetics of change in the expression levels after PHx or sham surgery (Fig. 2, Table 1). Three different patterns of expression levels were observed. Pattern a shows similar changes at 6 and 12 h after the surgical challenge. Pattern b depicts significant changes in expression levels at 6 h post surgery, while pattern c shows a significant change at 12 h post surgery. Three of the identified proteins are involved in lipid and energy metabolism: adipose differentiation related protein (ADRP; 366) and enoyl coenzyme A hydratase 1 (ECoAH-7; 1006; both pattern a), with notable up-regulation at 6 and 12 h, and malate dehydrogenase (MD; 549), with increased levels at 12 h (pattern c), in PHx and sham-operated mice. In the case of the first two proteins, the increase after PHx was greater than the increase after sham surgery (Table 1).

Seven proteins were found to be functionally related to intracellular signaling and cell proliferation: Selenium binding protein 2 (SBP2; 342) and poly (rC) binding protein 1 (517) were up-regulated at both 6 and 12 h after surgery (pattern a). However, in the case of SBP2, the increase was higher in the sham-operated mice than in the PHx mice (Table 1). Cytoplasmic gamma-actin (478) and a protein similar to annexin A4 (572) displayed an increased concentration when compared to both untreated (no surgery) and sham-operated mice at 6 h after treatment (pattern b). Finally, eukaryotic translation initiation factor 3 (TIF3; 526), *D*-dopachrome tautomerase (DDT; 911), and transcription factor Oct-3 (550) showed the most pronounced increases in concentration at 12 h after surgery (pattern c); in all three cases the increase in the PHx mice was greater than in the sham-operated mice (Table 1).

Three identified proteins are involved in protein and amino acid metabolism. While ATPase 3 (390) showed the most predominant change at 12 h after surgery (pattern b), the mouse homologue of rat p47 (identified as an unnamed protein product; 445) was up-regulated after PHx (pattern a). The Haao protein (600), was uniformly down-regulated after sham surgery and PHx (pattern a) (Table 1).

Three proteins were identified as acute-phase proteins: haptoglobin precursor (479), serum amyloid A (976), and serum amyloid A 2 (992) (all pattern a). All three showed appreciable increases after PHx and sham surgery, with a greater degree of up-regulation after PHx than after sham surgery Table 1). One protein, which was similar to amphoterin (733), showed a strong increase at 12 h after sham surgery but a decrease after PHx (pattern c). In summary, 12 out of 17 identified proteins were up-regulated after PHx. The remaining five proteins were either down-regulated after PHx or their expression levels were not significantly different from that of the sham animals.

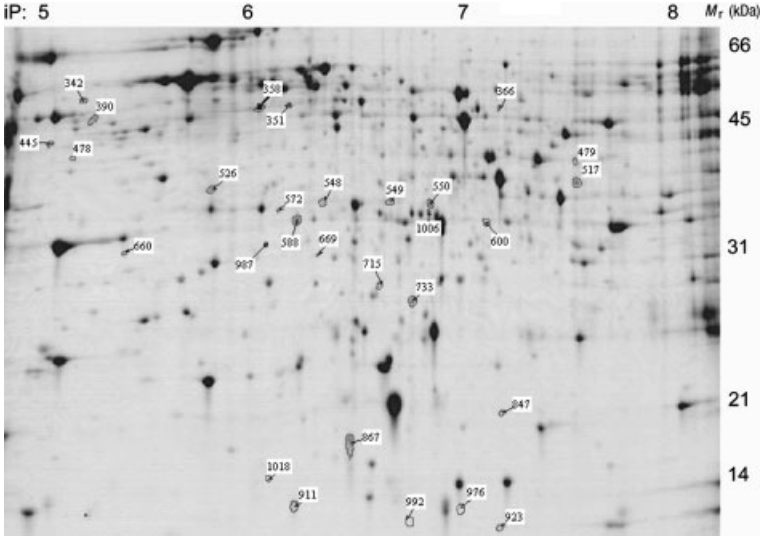

Figure 1. Reference 2-D gel map. All spots with significant changes in concentration after PHx or sham operation are highlighted. The identification numbers of the spots correspond to the numbers in Table 1. The linear range of p*I* extends from pH 5 (left margin of gel) to pH 8 (right margin). The numbers on the right are M_r (kDa).

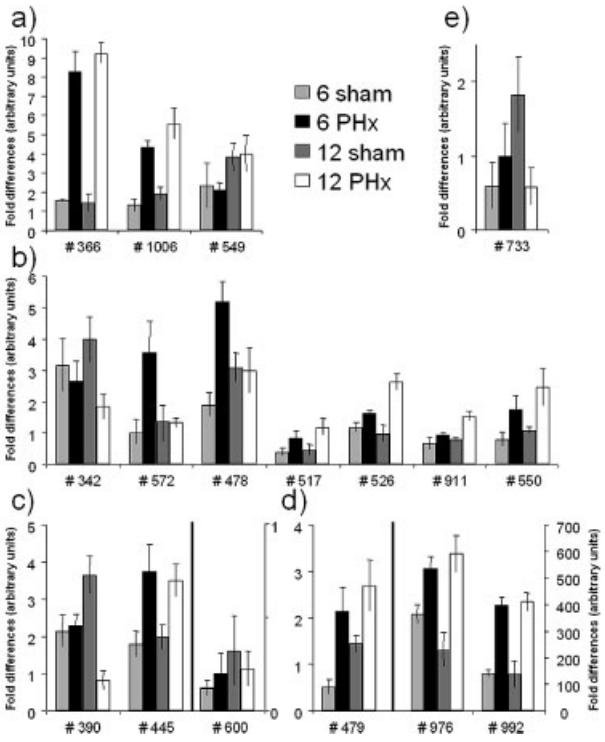

Figure 2. Concentrations of identified proteins with altered abundance after PHx and/or sham surgery. All panels show the fold changes (arbitrary units) of spot intensities of the indicated proteins at 6 and 12 h after surgery (sham or PHx) in comparison with the animal group without surgery. The grouping and numbering refers to the proteins listed in Table 1. (a) Lipid and energy metabolism, (b) intracellular signaling and cell proliferation, (c) protein and amino acid metabolism (note scale difference of Y-axis, left axis for 390 and 445, right axis for 600), (d) acute phase response (note scale difference of Y-axis, left axis for 479, right axis for 976 and 992), (e) protein up-regulated only after sham surgery. Each bar represents the mean of five separate gels of samples comprised from pooled tissue from five animals.

4 Discussion

Here we demonstrate that liver regeneration induced by PHx in mice is associated with liver proteome alterations. The wide-ranging approach that we have taken has made it possible to study the effects of different signaling and metabolic pathways on the liver proteome at the same time. Sample pooling (five animals *per* study group) and repetitive 2-D runs (four for each study group) was used to compensate for inter-gel variability, which is inherent to 2-DE [22]. Also, comparison of the PHx and corresponding sham groups helped to identify proteme changes that were specific to the regenerative process and not as a consequence of the surgical trauma itself (sham surgery). To our knowledge, this is the first description of specific liver proteome alterations after surgical reduction of liver volume that have been identified by 2-DE in combination with MS and protein identification *via* mass fingerprinting. The advantage of this broad-range proteomic methodology is that it allows for an unbiased approach to liver protein expression alterations after PHx. Therefore, it has the capacity to identify proteins that have not yet been associated with the process of liver regeneration by other methodological designs. Our study has identified several proteins whose expression was significantly altered by 70% liver resection. These proteins are involved in various metabolic pathways, including lipid and energy metabolism, intracellular signaling, protein and amino acid metabolism and the acute-phase response. In the following section, the possible functions of all these proteins, and especially those that showed changes after PHx, will be discussed.

4.1 Lipid and energy metabolism associated proteins

Two proteins associated with cellular metabolism, ADRP and ECoAH-1, were predominantly up-regulated after PHx. ADRP is ubiquitously expressed and is associated with lipid droplet storage. Its induction can be mediated by inflammatory stimuli [23] and results in lipid accumulation and lipid droplet formation [24, 25]. ECoAH-1 together with H_2O_2-generating fatty acyl-CoA oxidase and 3-ketoacyl-CoA thiolase is a constituent of the peroxisomal β-oxidation system [26–28]. This system is responsible for fatty acid catabolism. Defective or insufficient fatty acid catabolism causes steatohepatitis and increased proliferation of peroxisomes [29]. In this context, it is of interest to note that peroxisome proliferator-activated receptor α is necessary for cell cycle progression in regenerating mouse liver [30]. Moreover, it has been postulated that H_2O_2 overproduced by the sustained transcriptional activation of the peroxisomal β-oxidation system and concomitant cell proliferation contribute to hepatocarcinogenesis in livers with peroxisome proliferation [31].

Increased expression of proteins involved in lipid metabolism, and especially those, responsible for fatty acid catabolism, most likely represents an adaptive change in the hepatocytes. This adaptation can be viewed from various perspectives: The hepatocytes may acquire fatty acids as a substrate for the generation of high-energy metabolites [32] or phospholipids required for cytoplasmic membrane formation. It has also been suggested that alterations in fatty acid patterns could influence transmembrane signaling in relation to proliferative and apoptotic pathways in hepatocytes [33]. Furthermore, the relative increase in fatty acid and chylomicrons delivery from the gastrointestinal tract to the liver after PHx could contribute to increased lipid storage. Mild, histologically evident fatty changes do not seem to impair hepatocyte function. Histological analysis of a series of hepatecomized animals has revealed an accumulation of lipid droplets in the cytoplasm of hepatocytes at 44 h after PHx, without obvious impact on liver regeneration (data not shown). However, it has been noted that excessive hepatocyte steatosis is associated with impaired liver regeneration after PHx [34].

Taken together, these data suggest that increased lipid storage (ADRP) and fatty acid catabolism (ECoAH-1) are crucial aspects of the regenerative response after PHx. In this

Table 1. List of proteins with at least 2-fold alterations after PHx or sham surgery

No.	Protein name	% Sequence coverage	Function	Ratio	
				6 h PHx *vs.* sham	12 h PHx *vs.* sham
a) Lipid and energy metabolism					
366	adipose differentiation related protein	22%	lipid storage, trafficking	5.4	6.5
1006	enoyl coenzyme A hydratase 1	34%	lipid metabolism	3.3	2.9
549	malate dehydrogenase	28%	citric acid cycle component	0.9	1.0
b) Intracellular signaling and cell proliferation					
342	selenium binding protein 2	10%	cell growth regulation factor	0.8	0.4
572	similar to annexin A4	24%	DNA replication, cell proliferation, signaling	3.6	1
478	actin, gamma, cytoplasmic	18%	amino acid transport, implicated in LR	2.7	1
517	Poly(rC) binding protein 1	37%	mRNA binding	2.1	2.7
526	eukaryotic translation initiation factor 3	29%	TIF, cadmium-responsive proto-oncogene	1.4	2.7
911	D-dopachrome tautomerase	69%	pro-inflammatory signaling	1,4	1,94
550	transcription factor Oct-3	16%	Regulation of transcription	2,1	2,3
c) Protein and amino acid metabolism					
390	ATPase 3	25%	proteasome 26s subunit component	1	0.2
445	unnamed protein product	48%	homologue of p47-rat – membrane fusion mitotic Golgi fragments	2.1	1.8
600	Haao protein	34%	tryptophan metabolism	1.6	0.7
d) acute phase response					
479	haptoglobin precursor	34%	acute phase serum proteins	4.2	1.9
976	Serum amyloid A	74%		1.5	2.6
992	Serum amyloid A 2	62%		2.8	2.9
e) Protein up-regulated only after sham surgery					
733	similar to amphoterin	19%	high mobility group1, pro inflammatory signaling	1.7	0.3

Protein identification number, (No.) protein name, MS sequence coverage, protein function, and the ratios of the relative protein concentrations (2-D gel spot abundance, as compared to the values for the reference group without surgery) of the study groups are listed (quotients of PHx *vs.* sham animals at 6 and 12 h after surgery: ratio above 1 = up-regulation and ratio below 1 = down-regulation in PHx animals). The identified proteins are grouped according to their functions: (a) three proteins associated with lipid metabolism and energy metabolism; (b) seven proteins related to intracellular signaling and cell proliferation; (c) three proteins related to protein and amino acid metabolism; and (d) three acute-phase proteins up-regulated after PHx. (e) Spot 733 (bottom line, similar to amphoterin) was only altered under the condition of sham surgery.

context, the modulation of defective lipid metabolism on the basis of a pre-existing hepatic steatosis during liver regeneration might be of therapeutic value. Expression level changes, connected with lipid metabolism, have also been shown using microarray analysis after PHx [7, 8, 10]. ADRP, which is in line with our studies, was found to be up-regulated after PHx [7], while Stearyl-CoA desaturase [10], very-long-chain acyl-CoA synthetase [7], and carnitine octanoyl transferase [8], were all down-regulated. MD, an enzyme that participates in the citric acid cycle, showed similar alterations after PHx and sham surgery. This enzyme generates NADH, with the transformation of malate to oxaloacetate. The citric acid cycle is the final common catabolic pathway for the oxidation of fuel molecules and is therefore a critical source of energy for the cell. The up-regulation of MD as a result of both surgical challenges could illustrate its role among the basic cellular functions of the liver, which are needed in both cases of hepatic adaptation.

4.2 Intracellular signaling and cell proliferation associated proteins

The second functional group, related to signaling and cellular proliferation, that is altered after PHx includes SBP2, a protein similar to annexin A4, the gamma-actin, the poly (rC) binding proteins αCP-1 and αCP-2, TIF3, DDT and the embryonic transcription factor Oct-3. SBP2, which is thought to serve as an inhibitor of cell proliferation [35], was

found to be differentially regulated after PHx. The concentration of SBP2 showed a peak at 6 h after PHx and returned to lower expression levels at 12 h after PHx; at 12 h the increase after sham surgery was greater than that after PHx. On the basis of the finding that peroxisome proliferators lower the expression of SBP2 in mouse liver [35], SBP2 provides a connection between cell proliferation and lipid metabolism. In this respect we can hypothesize that peroxisome proliferation after PHx improves fatty acid catabolism and promotes cell proliferation by decreasing the level of the proliferation inhibitor SBP2.

The annexin protein family has already been implicated in liver regeneration [36–38]. Our data indicate that annexin A4 is up-regulated after partial hepatectomy. Annexins are ubiquitous and structurally homologous proteins with diverse biological activities [39]. They have been implicated in a broad range of cellular functions, including calcium-activated cellular signal transduction events [40, 41], DNA replication, cell proliferation, ion-channel formation, apoptosis [42] and vesicle aggregation and trafficking [43]. Moreover, annexin A4 appears to promote ethanol-induced cell lesions in association with NFκB activation [44, 45]. Gamma actin has also previously been described in the context of liver regeneration. It has been shown to accumulate next to the hepatocyte plasma membrane in rat livers during liver regeneration [46]. In the context of cell growth and proliferation, gamma actin might be involved in the regulation of amino acid transport [47], which is also up-regulated after PHx [48]. The poly (rC) binding protein belongs to the group of post-transcriptional control proteins. The two major cytoplasmic poly(C)-binding proteins are αCP-1 and αCP-2 [49]; the stabilizing function of these proteins is exerted upon actively translating target mRNAs [50]. Post-transcriptional regulation seems to be the predominant mode of control for most genes expressed at or beyond the G1 phase of the hepatocyte cell cycle [51–53].

TIF3 is a regulatory protein that was found to be up-regulated after PHx. The gene for TIF3 was also recently identified as a novel cadmium-responsive proto-oncogene [54]. By controlling translation initiation, the cells regulate the critical processes of growth and proliferation [55]. TIF3 has also been identified as a TGF-beta receptor binding protein. This is a group of proteins that interact with TGFβ type I receptors and play important roles in TGFβ signaling. TGFβ1 is a potent inhibitor of hepatocyte proliferation and an inducer of fibrogenesis and is produced by non-parenchymal liver cells during liver regeneration [56, 57]. DDT converts 2-carboxy-2,3-dihydroindole-5,6-quinone (*D*-dopachrome) into 5,6-dihydroxyindole and probably also functions as a pro-inflammatory signaling factor [58]. DDT is highly homologous and correlates in abundance with macrophage migration inhibitory factor, which is a cytokine for inflammatory T-cell activation and has a similar enzymatic activity [59]. Oct-3 has not been found to date in normal adult liver and has only been implicated in mouse embryogenesis [60]. Our observation of Oct-3 up-regulation after PHx, if confirmed by further experiments (currently underway), has very interesting implications. It is possible that Oct-3 could be reactivated in hepatocytes under the influence of proliferative stimuli associated with liver regeneration.

4.3 Protein and amino acid metabolism associated proteins

The expression of three proteins related to protein and amino acid metabolism, was altered in response to PHx: ATPase 3, a mouse homolog of rat p47, and the Haao protein. ATPase 3 brings into focus the potential role of the proteasome during liver regeneration, since it is an ATP binding subunit of the 26S proteasome. This ATP-dependent proteolytic protein complex prevents the accumulation of non-functional, misfolded and potentially toxic proteins in eukaryotic cells. The important protective role of the 26S proteasome has been demonstrated under the influence of cellular stress factors (*e.g.* heat shock, oxidative stress) and in a variety of disease states (*e.g.* cystic fibrosis and major neurodegenerative diseases) [61]. Moreover, inhibition of proteasome function in hepatocytes leads to apoptotic cell death [62]. Liver regeneration can be regarded as a strong cellular stress factor, and it is therefore reasonable to imagine a role for proteasome function in the prevention of liver failure after PHx. A link to the regulation of the proteasome after PHx has also been also described by microarray experiments in which it was found that the 55.11 binding protein mRNA, a regulatory component of the 26S proteasome, is up-regulated after PHx [7].

Spot 445 was identified as an unnamed protein product, which proved to be homologous to rat p47, an accessory protein for the p97-mediated fusion pathway. P97 is known to be involved in the heterotypic fusion of transport vesicles with their target membranes and the homotypic fusion of membrane compartments, and it is essential for the regrowth of Golgi cisternae from mitotic Golgi fragments [63]. The Haao protein, which showed similar alterations after PHx and sham surgery, is involved in the metabolism of tryptophan [64]. The relevance of tryptophan has been shown for cell proliferation and immunological functions in the context of T-cell regulation. Furthermore, tryptophan can act as a cell toxin in higher concentrations and contributes to the pathomechanisms of liver failure. The up-regulation of the Haao protein might therefore reflect an adaptive mechanism of the liver to improve its detoxification properties, a function that is relevant for various stress situations including liver regeneration.

4.4 Acute-phase response associated proteins

The initiation of an acute phase response after PHx is a well-known phenomenon [5, 65] and the up-regulation of acute phase mRNAs post PHx has been well documented in various microarray experiments [7, 8, 10]. In the present study, we observed the up-regulation of three acute phase proteins

after PHx but not after sham surgery. Haptoglobin precursor displayed an increase after PHx or after sham surgery. In contrast, serum amyloid A and serum amyloid A2 were upregulated after both PHx and sham surgery. However, PHx resulted in a comparatively stronger induction of these two proteins indicating a more pronounced acute-phase response in comparison with sham surgery. Additionally there seems to be also a qualitative difference of the acute-phase responses due to differential regulation of the involved acute-phase response proteins, which is reflected by the lack of increase of the haptoglobin precursor after sham surgery.

5 Concluding remarks

In summary, our data illustrate the enormous complexity of the process of liver regeneration. The changes in the expression of several proteins that we have documented after PHx reflect the involvement of various cellular metabolic pathways. Our results point to several cellular functions, not yet studied in the context of liver regeneration, as valuable targets for further investigation. Among these are peroxisome activity, which could provide a platform for the cellular energy supply through lipid catabolism and the regulation of regeneration (*e.g. via* SBP2). As indicated by the changes in ATPase 3 with PHx, another functional complex, that could be of relevance for liver regeneration is the proteasome. Furthermore, specific catabolic pathways such as tryptophan catabolism and various intracellular signaling factors and proteins involved in intracellular trafficking are affected by liver regeneration. Future investigations will focus on the specific functional role of identified proteins in regenerative response after PHx.

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