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Gene expression profile in the regenerating rat liver after partial hepatectomy

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***Background/Aims*: When a loss of hepatic mass occurs, the expression of a large number of genes is either induced or altered, accompanying hepatocyte proliferation. In the present study, we made an in-house cDNA microarray containing 4608 elements (Liver chip), and analyzed extensively gene expression profiles of the regenerating liver after 70% partial hepatectomy (PHx) in rats.**

***Methods*: RNAs were prepared from three rat livers at each time point (taken at 0, 6, 12, 18, 24, 48, 72 h, and 1 week after PHx). Using the liver chip, we performed large-scale analysis of gene expression during liver regeneration. Elements either up- or down-regulated more than twofold at one or more time points were selected.**

***Results*: Among the 4608, 382 were identified. Using cluster analysis, we found great similarity between gene-expression profiles at 12 and 18 h after PHx as well as between 48 and 72 h after PHx. We also found that there are at least six distinct temporal patterns of gene expression in the regenerating rat liver after PHx.**

***Conclusions*: These results indicated that microarray analysis is a powerful approach for monitoring molecular events in the regenerating liver.**

***Keywords*: Complementary DNA microarray; Liver regeneration; Hepatectomy; Clustering analysis**

1. Introduction

Hepatocytes rarely divide in the normal healthy liver. However, their proliferative capacity and the ability of the liver to adapt to variable metabolic demands are quickly displayed when liver mass increases or decreases. PHx, resulting in the removal of approximately 70% of the liver, is widely utilized for studies of liver growth in experimental animals. The regenerative response is proportional to the amount of liver removed [1]. This demonstrates that the liver can regulate its own growth and mass, and that signals from the body can have negative as well as positive effects on liver mass until the correct size is reached. Knowing when and where genes are expressed provides a strong clue as to its biological role. Comprehensively, the pattern of genes expressed in a cell can provide detailed information about its state. Although many investigations of the genetic alterations responsible for liver regeneration had been reported, it was difficult to analyze them extensively. With the advent of cDNA microarray technology, genomewide expression of thousands of genes can be simultaneously

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Abbreviations: apo, apolipoprotein; AldB, aldolase B; ADH, alcohol dehydrogenase; GOT, glutamic-oxaloacetate transaminase; Hsp70, heat shock protein 70; IL-6, interleukin-6; PHx, partial hepatectomy; PPARα, peroxisome proliferator-activated receptor α; TNF, tumor necrosis factor; SULT1B1, thyroid hormone sulfotransferase.

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analyzed, facilitating differential expression monitoring of a large number of activated or suppressed genes under various biological conditions. In the present study, at several time points after PHx, RNAs were separately isolated from three rat livers and hybridized with a cDNA microarray containing 4608 elements made from rat livers. Using it, we performed a large-scale analysis of gene expression during liver regeneration.

2. Materials and methods

2.1. Rats

Male Sprague–Dawley rats, aged 10–12 weeks, were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). All rats used were maintained under specific pathogen-free conditions in our animal facility. Animal care was in accordance with the guidelines of the National Research Institute for Child Health and Development.

2.2. Partial hepatectomy

The partial hepatectomy procedure was performed under sterile conditions by the method described previously [3]. This resulted in removal of 70% of the total liver.

2.3. Sampling

We killed three rats at 6, 12, 18, 24, 48, 72 h, and 1 week after PHx. The livers were weighed, and the amount of glutamic-oxaloacetate transaminase (GOT) in serum was measured. Liver blocks up to 1 cm^3 were embedded in OCT compound (Tissue-Tek, Elkhart, IN) and snap-frozen in isopentane. Frozen sections were then cut at 6 μm in a cryostat for immunohistology. A second part of the liver was immediately snap-frozen for subsequent molecular analysis, and a third part was fixed in 10% neutral buffered formalin for routine histology.

2.4. RNA Isolation

We extracted the total cellular RNA from frozen liver tissue using ISOGEN (Nippon gene, Tokyo) as directed by the manufacturer. Briefly, a small piece of liver tissue (<1 cm^3) was lysed by addition of denaturing solution containing phenol and guanidinium isothiocyanate/cationic detergent, followed by phenol–chloroform extraction and isopropyl alcohol precipitation. We confirmed the RNA quality using a lab chip system (Agilent Technologies).

2.5. Construction of a cDNA microarray ('liver chip') with a normalized liver cDNA library

Ordinary cDNA libraries contain a high frequency of undesirable clones because of the redundancy of mRNA species in the cell. To exclude redundancy from the liver cDNA library, it was normalized by the subtraction method using drivers as described previously [4–6]. Sequencing of the clones was performed using the MegaBACE 1000 DNA Sequencing system (Amersham-Pharmacia Biotech). cDNA fragments amplified by polymerase chain reaction (PCR) were spotted onto glass slides using a robotic mechanism. A total of 4608 clones were printed on our liver chip. A preliminary series of experiments with the liver chip showed that this cDNA microarray contained many genes that were specifically and highly expressed in the liver, compared to other tissues. Also, about 80% of the arrayed clones were found to be successfully hybridized with target cDNAs, indicating that this cDNA microarray is efficient for monitoring tissue-specific gene expression. Sequence homology was confirmed by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

2.6. Preparation of fluorescence-labeled cDNA and microarray hybridization

RNA prepared from rat livers before hepatectomy was used as a reference for all cDNA microarray analyses. Ten micrograms of each extracted total RNA was reverse transcribed with Cy3- and Cy5-conjugated dUTP (Amersham-Pharmacia Biotech) using Super script II reverse transcriptase (Life Technologies). After 2 h incubation, labeled probes were concentrated in a Microcon filter device (Millipore), diluted in 15 μl hybridization solution [3.4 × SSC containing 0.3% SDS, 20 μg poly(A)DNA, and 20 μg yeast tRNA], and applied to the microarray.

2.7. Hybridization and scanning

We hybridized labeled targets against cDNA microarrays with overnight incubation at 65 °C. The slides were washed twice with 2 × SSC containing 0.5% SDS for 5 min at room temperature, once with 0.2 × SSC containing 0.5% SDS at 40 °C for 3 min, and finally with 0.2 × SSC for 3 min. Hybridized images were scanned by a fluorescence laser scanning device, GenePix 4000A (Axon Instruments, Inc., Foster City, CA). At least three hybridizations were performed at each time point.

2.8. Data analysis

The signal intensities were quantified by GenePix Pro 3.0 software (Axon Instruments, Inc., Foster City, CA) Subsequently, we normalized the obtained numerical data with classical linear regression techniques. In brief, quantified signal intensities were converted by taking logarithms of base two. Using transformed data derived from each pair of competitive hybridization images, we drew scatter diagrams to compare sample signal intensities with those derived from controls, and executed regression analysis. The given residuals explain logarithmic gene expression ratios. Therefore, we selected genes whose average residuals were more than 1 or less than -1, i.e. representing a twofold difference in expression level.

To analyze the selected gene expression data, we applied *k*-means cluster analysis. Preceding the *k*-means cluster analysis, we performed hierarchical clustering to appraise the number of groups. Euclidean distance was used as the dissimilarity measure. Whole analyses were executed with Microsoft Excel (Microsoft, Redmond, WA), R (http://www.r-project.org/) and GeneSpring (Silicon Genetics, San Carlos, CA).

2.9. Semiquantitative RT-PCR

Total RNA was reverse transcribed, diluted and used for PCR. We amplified cDNA fragments for 25, 30, and 35 cycles. PCR primers were designed on the basis of the reported cDNA sequences. The β-actin gene was used as a positive control. Templates without reverse transcription were amplified as negative controls.

2.10. Immunohistological examination

One hour before sampling, all rats received an i.p. injection of BrdU (100 mg/kg, dissolved in distilled water). Liver samples were snap-frozen in liquid nitrogen and stored at −80 °C until they were sectioned on a cryostat. The sections were air dried and fixed in acetone at −20 °C overnight, followed by air drying for 1 h. After 1 h of incubation with a mouse anti-BrdU biotin-conjugated monoclonal antibody (Chemicon, Temecula, CA), diluted 1:50 in PBS solution containing 2% bovine serum albumin and 0.1% sodium azide, the secondary antibody, anti-mouse IgG conjugated to alkaline phosphatase (Sigma, St. Louis, MO) diluted at 1:80 in the above working solution, was added and incubated for another hour. Color development was done with a Vector Red detection reagent kit (Vector Laboratories, Inc., Burlingame, CA). Finally, we counterstained the sections with hematoxylin (Sigma, St. Louis, MO). The ratio of the positive stained area to the whole area (BrdU index) was calculated in each sample in a randomly chosen appropriate field of 200 magnifications using a FUJIX

HC-2000 (Fuji Film, Tokyo) interfaced with Mac SCOPE/PPC software. The mouse monoclonal anti-β tubulin antibody (Sigma), diluted 1:300, was used for staining β tubulin in the cryosections from the reference and experimental livers at various time points after hepatectomy.

2.11. Statistical analysis

Statistical analysis of the data was performed by Student's *t*-test. $P < 0.05$ was considered statistically significant.

2.12. Ethics

We conducted all our experimental procedures in accordance with the policies of the Animal Ethics Committee of the National Research Institute for Child Health and Development.

3. Results and discussion

Liver regeneration models have been widely used to study cell cycle events because they provide a setting in which a large percentage of cells undergo a synchronized round of proliferation. To define the relevant parameters in this model system, we measured the weight of the regenerating liver as well as the index of incorporation of BrdU into hepatocytes at the various time points after surgery. As shown in Fig. 1, BrdU stained strongly 24 h after PHx (Fig. 1A). The time course of BrdU incorporation was calculated by the ratio of the positive stained area to the whole area in each sample in a randomly chosen appropriate field, and showed a significant ($P < 0.05$) increase 24 h after PHx (Fig. 1B). A ratio of liver to whole body was significantly increased, and liver weight was recovered to the pre-hepatectomized level by 1 week. The amount of serum GOT increased transiently 1 day after PHx, and decreased subsequently (Fig. 1C). We identified 382 elements that altered by more than a twofold intensity at least one time point (Fig. 2A). We undertook hierarchical clustering of eight time points using GeneSpring software. Based on the similarity of the expression profile of genes presented by the dendrogram, time points showing the most similar patterns of gene expression were located next to each other and placed in a major branch of the dendrogram. Twelve- and 18-h as well as 48- and 72-h patterns were clustered together as separate groups in major branches,

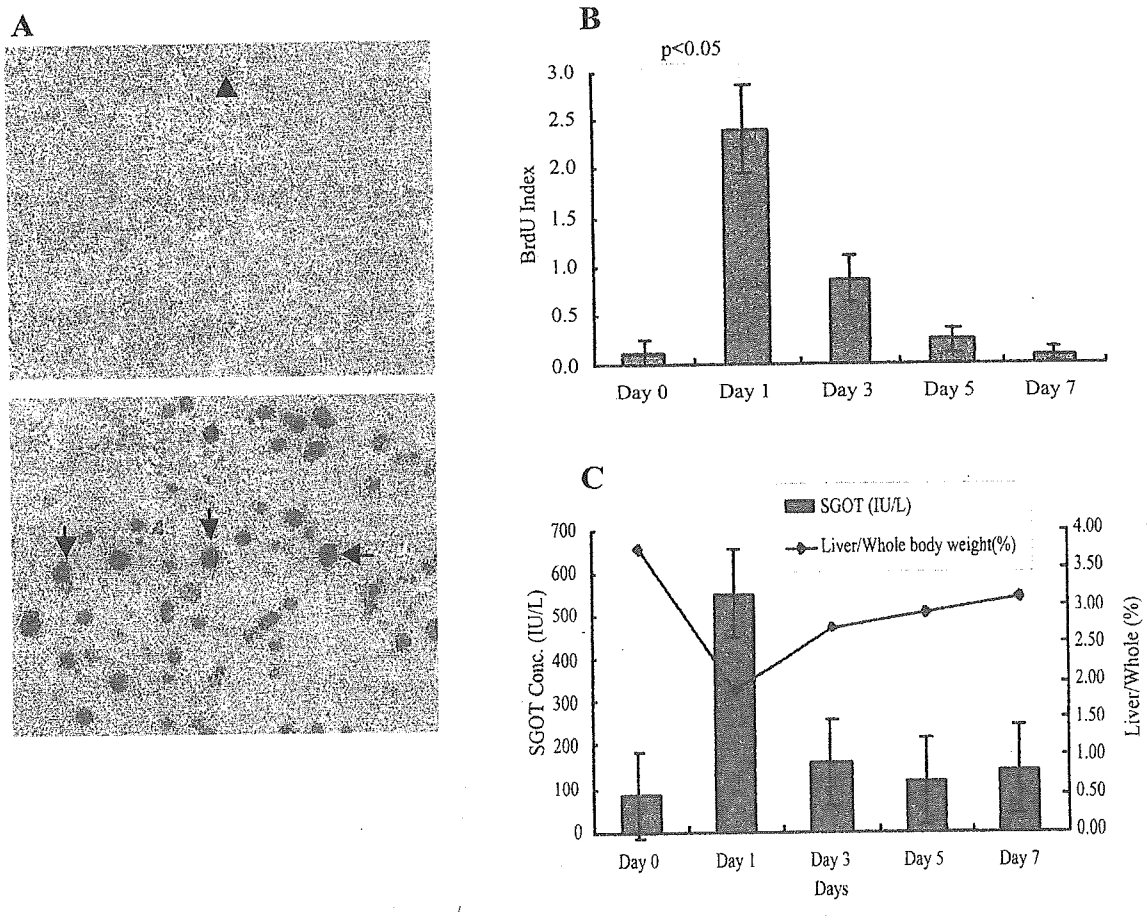

Fig. 1. Immune-staining of BrdU and time course of changes in the BrdU index, percent regeneration, and serum GOT after PHx. (A) BrdU was stained strongly at 24 h (A-b) after PHx compared with the control (A-a). (B) The ratio of the positive stained area to the whole area (BrdU index) was calculated in each sample in a randomly-chosen appropriate field. The BrdU index was significantly ($P < 0.05$) increased 24 h after PHx. C. The percent of liver regeneration (liver/whole body) after PHx significantly increased, and recovered to the pre-hepatectomy level by 1 week. The amount of GOT in serum increased transiently 1 day after PHx, and decreased subsequently.

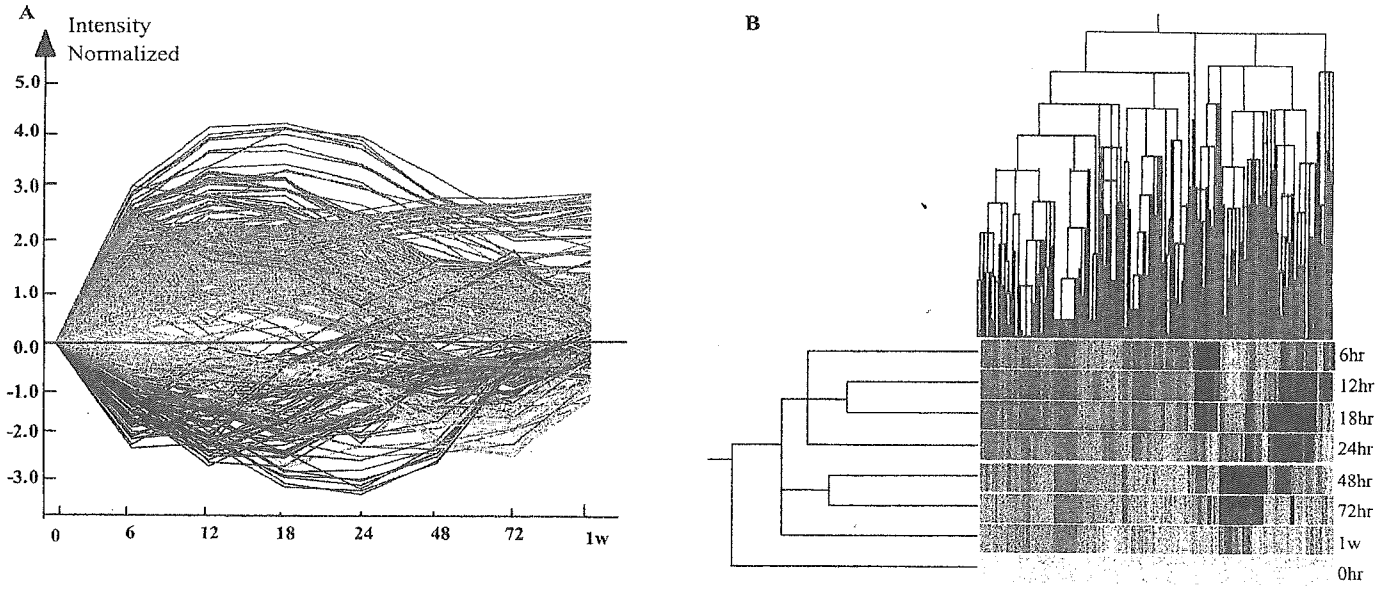

Fig. 2. Hierarchical cluster analysis of 382 selected elements. (A) Three hundred and eighty-two elements differing by more than twofold intensity at least one time points were identified. (B) A hierarchical clustering of eight time points; 12 and 18-h as well as 48- and 72-h time points were clustered together as separate groups in major branches, indicating that these time points share common expression profiles of genes, respectively.

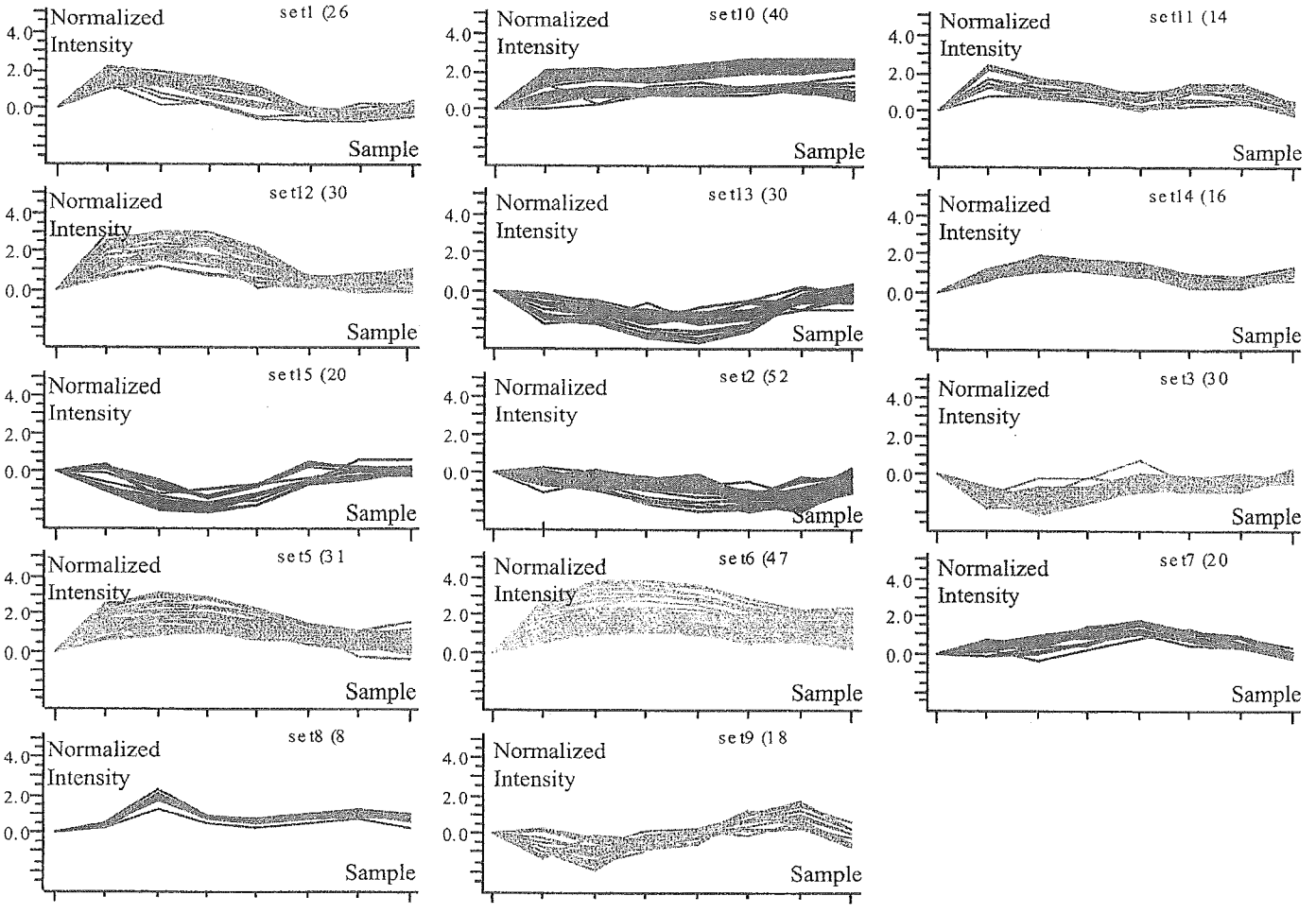

Fig. 3. Cluster analysis of differentially-expressed genes identified by cDNA microarray-based gene expression profiling. The K-means method was used and these genes were classified into 14 clusters.

indicating that these time points share common expression profiles of genes (Fig. 2B). DNA replication after PHx starts approximately 10–12 h later and reaches a maximum at 24 h, and that a second smaller peak of DNA replication occurs at 36–48 h in rats [2]. Our findings enable us to categorize time points into four phases: 6 h (before hepatocytes start replication; immediate early phase); 12–18 h (when hepatocytes start replication; early phase); 24 h (when replication reaches a peak; intermediate phase); and at 48–72 h (when a second round of replication occurs; late phase). Therefore, we suggest our findings and previous reports [2] are not contradictory. We found that 382 elements showed altered levels of expression consistently in all three regenerating rat livers tested at each time point. To facilitate the visualization and interpretation of the gene expression program represented in this very large body of data, we have used the method of *k*-means to order genes on the basis of similarities in their expression patterns and display the results in a compact graphical format (Fig. 3). We then categorized the selected elements into six distinct temporal induction patterns (Fig. 4).

Among the 382 elements, those regarded as the same genes were excluded as a result of evaluating their DNA sequence information and patterns of gene expression after PHx. Of the 116 genes thus selected, 65 were up-regulated, and 51 down-regulated. We categorized the 65 up and 51 down-regulated genes by the time points at which they attained maximum up or down-regulation, respectively (Tables 1 and 2). Of these 116 genes, 40 had been previously reported to be involved in liver regeneration [1,2].

3.1. Rapid transient induction

Twenty-seven genes were up-regulated immediately and reached maximum level within 6 h. Subsequently, they decreased gradually to basal levels by 48–72 h. Liver regeneration can be divided into two phases, priming and cell cycle progression. Priming, that is gene activation, is required for hepatocytes to progress to the G1 phase. It is expected that genes associated with priming exist in this category. On the other hand, priming is a reversible process initiated by cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6) [2]. In hepatectomized regenerating liver, it is known that this signaling pathway follows the sequence TNF → TNFR-1 → NFκB → IL-6 → STAT3 [2]. Among the genes in this category, angiotensinogen is already known to be induced by TNF → NFκB [7] and the IL-6 → STAT3 [8] signaling pathways. Apolipoprotein A-V, reported to be associated with an early phase of liver regeneration, and with maximal expression in the rat liver and its gene product in rat plasma 6 h after PHx [9], was also placed in this category.

3.2. Early induction

One hundred and twenty-two genes were the most strongly induced 12–18 h after PHx, and sustained their expression levels through the rest of the time course examined. In early G1, the cells fully respond to growth factors and can then progress through the cell cycle, overcome the G1 restriction point and undergo DNA replication. The

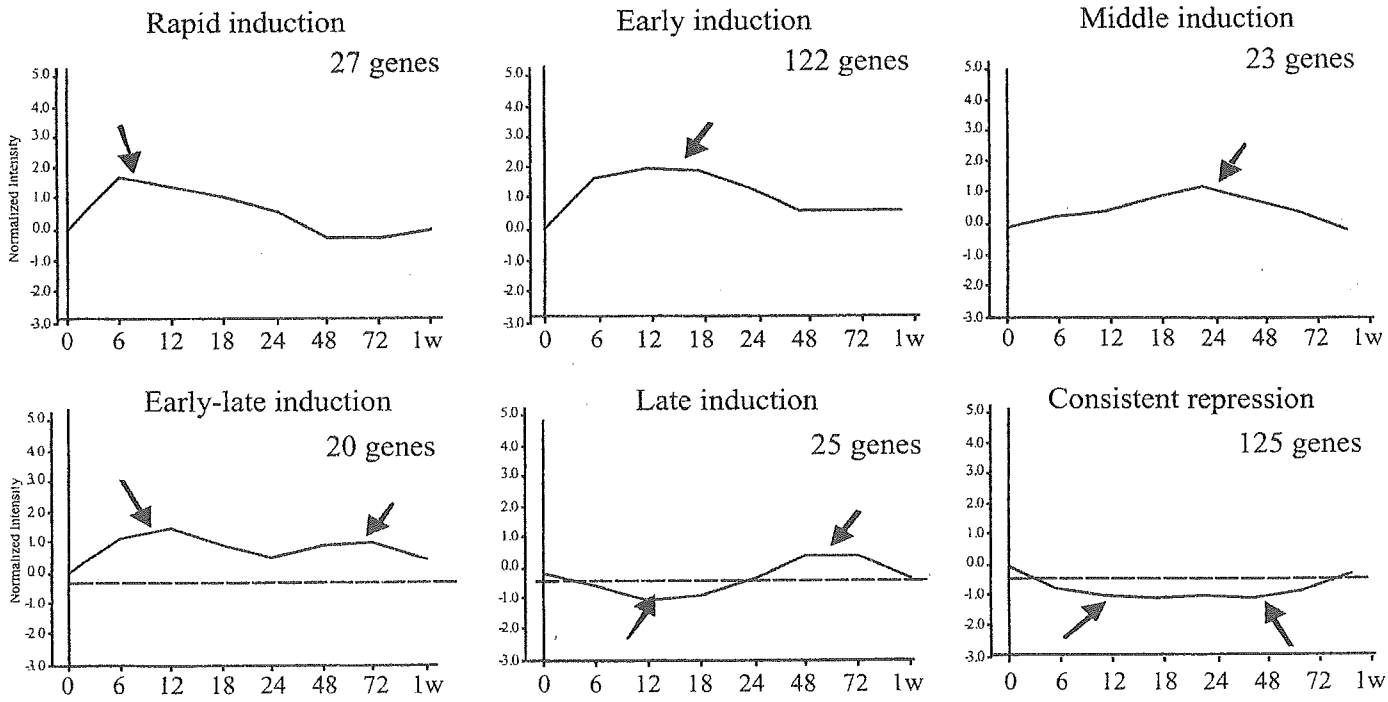

Fig. 4. Categorizing the selected elements into six distinct temporal patterns. The six patterns were designated Rapid induction, Early induction, Intermediate induction, Early–late induction, Late induction, and Consistent repression, on the basis of the results of the cluster analysis.

Table 1
Genes upregulated more than twofold after partial hepatectomy

	Gene description	Fold change		Gene description	Fold change
				Prepro-aspartyl protease	3.8
6 h	type-1 keratin intermediate filament	5.2		Tyrosine aminotransferase (Tat)	3.5
	Cytokeratin-18	4.4		Inter-alpha-inhibitor H4 heavy chain	3.2
	Actin	2.4		Amyloid A	3.2
	Lysine oxoglutarate reductase	5.0		Acetyl-Co A acetyltransferase	3.0
	Angiotensinogen	4.3		Complement component 3	2.5
	Pex14	3.6		Complement component 4 binding protein, beta	2.3
	Hepatocyte nuclear factor 6 beta	3.1		Stch	2.4
	Argininosuccinate lyase	2.9		Stress-associated endoplasmic reticulum protein 1	2.3
	Apolipoprotein A-V	2.7		PolyA, binding protein	2.3
	eIF-4A	2.7		eIF-4A, nuclear protein	2.3
	AldB gen repressor,	2.7		Cyp4b1	2.2
	Npm1	2.4			
	Glutaminyl-tRNA synthetase	2.5			
	Nucleotidase	2.3	24 h	RNA polymerase II -like	3.2
	Flavin containing monooxygenase 3	2.3		Tubulin	3.1
	Nucleic acid binding factor pRM10	2.2		Heat-shock-like protein	2.7
				Importin alpha	2.5
				Immunoglobulin light chain	2.5
12–18 h	Alpha 1-acid glycoprotein	12.2		Ribonucleotide reductase M1	2.2
	Alpha-1-macroglobulin	2.5		Histon	2.2
	Regeneration-associated serpin-1	2.4			
	T-kininogen	10.8			
	K-kininogen	4.6	48–72 h	Cytokeratin 8 polypeptide	2.6
	Contrapsin-like protease inhibitor	7.1		Thyroid hormone sulfotransferase	2.5
	Beta-myosin heavy chain	6.8		Apoxide hydrolase	2.4
	Hemopexin, pexin gene family	5.1		Diazepam binding inhibitor	2.3
	Fibrinogen	5.0		Lactate dehydrogenase	2.3
	Fibronectin 1	3.2		Glutathione S-transferase Yb-1 subunit	2.2
	Nuclei, microtubule-associated protein	3.3		Alcohol dehydrogenase	2.9
	Class 1 collagenase	2.9		tal-1	2.3
	Calreticulin	2.5		Hepatic (hepatocyte) nuclear factor 4	2.2
	Haptoglobin	4.2		ATP synthase	2.2
	Phosphoenolpyruvate carboxykinase	3.9			
	Ribosomal protein S29	4.6			
	Ribosomal protein L32	3.9	1 week	Caruloplasmin	3.5
	Ribosomal protein S6	2.5			
	Ribosomal protein L13	2.3			

nuclear protein B23 classified in this category was correlated with entry and progression through the S-phase [10]. Several acute phase proteins such as Haptoglobin, alpha 1-acid glycoprotein, inter-alpha-inhibitor H3, T-kininogen, amyloid A, and fibrinogen showed an early induction pattern just as previous reports [11–15]. The hemopexin gene was also found markedly induced as well as the previous report [16]. Heme-hemopexin is also believed to participate in cell growth and division [17]. The levels of expression of the mRNAs for several extracellular matrix proteins, including fibronectin as well as fibrinogen, also increased. Ribosomal proteins (S6, L4, S29, L13, L32) and serin protease inhibitors such as regeneration-associated serpin-1, rasp-1, and contrapsin-like protease inhibitor-related protein (Cpi-26) were also assigned to this category. Several genes associated with apoptosis such as Stch, the aforementioned ribosomal protein L4, and S29 were also included in this category.

3.3. Intermediate induction

Twenty-three genes were strongly induced 24 h after PHx, a time corresponding to the S phase. We classified the M1 subunit of ribonucleotide, tubulin, histone, and importin alpha into this category. The expression of the M1 subunit of ribonucleotide increases near the end of G1, and decreases at the end of S phase [18]. Microtubules such as β tubulin play an essential role in cell division, and some of the β tubulin isotype transcripts were found more highly expressed in dividing than in resting cells [19].

Table 2
Genes repressed less than 0.5 after partial hepatectomy

Time	Gene description	Fold change	Time	Gene description	Fold change
6 h	Glucose-6-phosphatase catalytic subunit		24 h	Stearyl-CoA desaturase	0.2
	retSDR1	0.3		Thyroid hormone induced hepatic product	0.2
	Dolichyl-phosphate beta-glucosyltransferase (ALG5)	0.4		Cyp2c	0.3
	Bile acid-CoA-amino acid *N*-acyltransferase	0.4		CYC1	0.3
	Hydroxysteroid sulfotransferase	0.4			
		0.5			
			48–72 h	Carbonic anhydrase III	0.2
				Cyp2c	0.3
12–18 h	Thyroid hormone induced hepatic product	0.2		Apolipoprotein A-II	0.3
	Ndr2	0.2		Apolipoprotein A-IV	0.4
	RNase	0.3		Apolipoprotein C-I	0.5
	Long-chain acyl-CoA synthetase	0.3		Apolipoprotein C-III	0.5
	Fatty acid desaturase	0.3		Apolipoprotein C-IV	0.3
	Delta-4-3-ketosteroid 5-beta-reductase	0.3		Contrapsin-like protease inhibitor	0.4
	Taube nuss	0.3		Plasma proteinase inhibitor alpha-1-inhibitor III	0.4
	Alcohol dehydrogenase	0.3		Serine protease inhibitor	0.4
	A receptor for vasopressin-related Lys-conopressin	0.3		Kallikrein-binding protein	0.5
	RFLP marker	0.3		Adrenodoxin reductase	0.4
	Corticosteroid binding globulin	0.3		Glycine methyltransferase	0.4
	PD-1alpha, nucleotide pyrophosphatase	0.3		Dolichyl-phosphate beta-glucosyltransferase (ALG5)	0.4
	Estrogen sulfotransferase	0.3		Ornithine aminotransferase	0.4
	Glutathione S-transferase	0.4		Mitochondrial ribosomal RNA	0.4
	Phgdh gene for 3-phosphoglycerate dehydrogenase	0.4		Phenylalanine-tRNA synthetase	0.4
	Phosphatidyl-choline-dependent enzyme	0.4		Pyruvate kinase	0.4
	Glutathione reductase	0.4		Estrogen sulfotransferase	0.4
	Dolichyl-phosphate beta-glucosyltransferase (ALG5)	0.4		Succinyl-CoA synthetase	0.4
	Glycine methyltransferase	0.4		Alpha-2u-globulin (S type)	0.4
	Pyruvate carboxylase	0.5		Hemoglobin, alpha 1	0.4

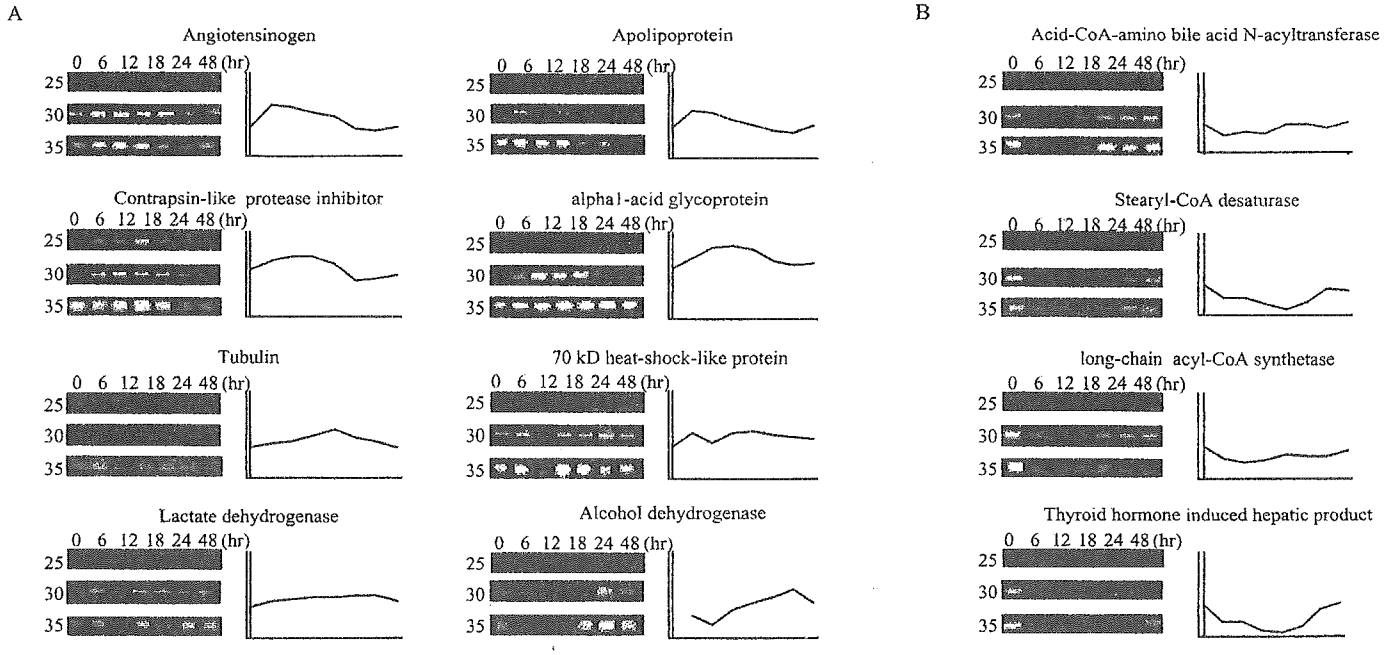

Fig. 5. Semiquantitative RT-PCR analysis of 12 genes, the expression of which was markedly altered and which are thought to be related to liver regeneration. Liver RNA derived from control and hepatectomized rats were used. cDNA fragments were amplified for 25, 30, and 35 cycles. The β-actin gene was used as a positive control. Templates without reverse transcription were amplified as negative controls.

Histone gene expression is coordinated with the cell cycle, and the identification of histone mRNA appears to be a reliable marker of the S-phase fraction [20]. Importin alpha is related to the induction of spindle formation in M phase [21]. These suggest the intermediate gene induction pattern is related to the S and M phases.

3.4. Early to late induction

Twenty elements showed an early–late pattern of induction. As with the early genes, they were initially induced during the first 6–12 h after PHx, but they were distinguished from the early genes by an additional increase in transcript levels measured at 48–72 h. The expression of these genes is thought to vary according to cell cycle position. Genes for the cytoskeleton, such as cytokeratin 8, 18, and keratin complex 1 were assigned to this category. Tyrosine aminotransferase, which was reported to increase in activity after PHx and manifest as two peaks [22], also showed an early–late induction pattern.

3.5. Late induction

Twenty-five genes down-regulated initially were then up-regulated again between 48 and 72 h after PHx. This interesting induction pattern may be linked to anti-proliferation control by mechanisms such as those involving apoptosis. Alcohol dehydrogenase (ADH), epoxide hydrolase, and glutathione S-transferase Yb-1 belong to this category. The ADH enzyme family may function in the metabolism of retinal. ADH-IV participates in the initiation of retinoid signaling by functioning as a retinol dehydrogenase [23]. It is known that retinoic acid (RA), injected within 4 h of PHx, inhibits DNA synthesis in regenerating liver [24]. In that paper, RA was shown to exert anti-proliferative activity accompanied by the repression of c-fos and c-jun expression and induction of apoptosis.

3.6. Consistent repression

Transcripts from 125 elements were repressed in the course of liver regeneration. This category included liver-specific genes such as those involved with metabolism of drugs, steroids, etc. The hydroxysteroid sulfotransferases (SULT2) play critical roles in drug metabolism. Delta-4-3-ketosteroid 5-beta-reductase is related to metabolism of steroid hormones. Bile acid-CoA-amino acid *N*-acyltransferase (BAT), a liver enzyme that catalyzes the conjugation of bile acids with glycine or taurine, was reported to decrease at 6 and 12 h in the regenerating liver [25]. Using our chip, the minimum BAT mRNA expression level was observed 6 h after PHx. The acute phase response (APR) is associated with decreased hepatic expression of many proteins involved in lipid metabolism. Due to the up-regulation of APR during first 12–18 h after PHx, the genes related to lipid metabolism are expected to decrease. The nuclear hormone receptor, peroxisome proliferator-activated receptor α (PPARα) plays key roles in regulation of hepatic lipid metabolism. Acyl-CoA synthetase, a well-characterized PPAR α target, was assigned to this category. Stearyl-CoA desaturase, an iron-containing enzyme that catalyzes a rate-limiting step in the synthesis of unsaturated fatty acids, was also included. Hepatic apolipoprotein A-II, A-IV, C-I, C-III, C-IV mRNA levels decrease 48–72 h after PHx. Hepatic apo C-III mRNA levels were reported to decrease to a minimum value of 46% of normal 72 h after the induction of inflammation [26].

To confirm the microarray analysis with the liver chip, we performed semiquantitative RT-PCR analysis of 12 genes, which were markedly altered at various time points and which are thought to be related to liver regeneration. Liver RNA derived from control and hepatectomized rats were used. As shown in Fig. 5, the results of the RT-PCR correlated well with those obtained using the liver chip. Additionally, we confirmed gene expression at the protein

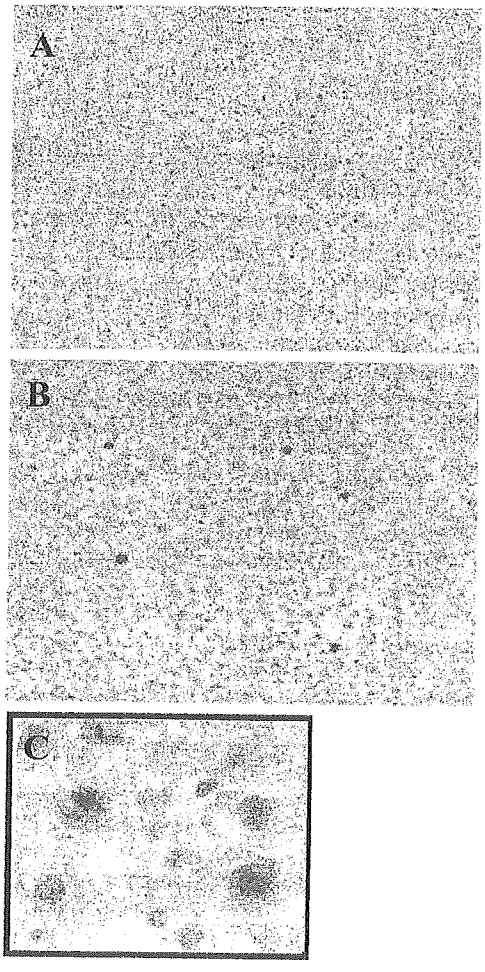

Fig. 6. Immunohistological staining of β tubulin. The mouse monoclonal anti-β tubulin antibody, diluted at 1:300, was used for staining β tubulin in cryosections from the control and experimental livers at each time point after PHx. We present the control (A) and an example 72 h after PHx (B), which were stained most strongly. Cells in mitotic phase were stained (C).

level by immunohistological staining. Fig. 6 shows staining of β tubulin from the reference and experimental livers at each time point after PHx by mouse monoclonal anti-β tubulin antibody. β tubulin was most strongly stained 72 h after PHx. β tubulin is in the intermediate induction category. Taking account of the time period for translation, these data are therefore not contradictory.

In summary, we have made an in-house cDNA microarray that are highly and specifically expressed in the liver. Using the liver chip, we performed large-scale analysis of gene expression during liver regeneration; 382 elements showed altered levels of expression (more than twofold up- or down-regulation) at specific time points. Cluster analysis showed strong correlations between expression patterns at 12 and 18 h, as well as between 48 and 72 h after PHx. Second, we categorized the selected elements into six distinct temporal patterns of induction. These results indicated that cDNA microarray analysis is a powerful approach for monitoring molecular events in a disease of which the pathogenesis is unknown.

Acknowledgements

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Stat3 protects against Fas-induced liver injury by redox-dependent and -independent mechanisms

See the related Commentary beginning on page 978.

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Signal transducer and activator of transcription-3 (Stat3) is one of the most important molecules involved in the initiation of liver development and regeneration. In order to investigate the hepatoprotective effects of Stat3, we examined whether Stat3 protects against Fas-mediated liver injury in the mouse. A constitutively activated form of Stat3 (Stat3-C) was adenovirally overexpressed in mouse liver by intravenous injection, and then a nonlethal dose of Fas agonist (Jo2) was injected intraperitoneally into the mouse (0.3 μg/g body wt). Stat3-C dramatically suppressed both apoptosis and necrosis induced by Jo2. In contrast, liver-specific Stat3-knockout mice failed to survive following Jo2 injection. Stat3-C upregulated expression of FLICE inhibitor protein (FLIP), Bcl-xL, and Bcl-2, and accordingly downregulated activities of FLICE and caspase-3 that were redox-independent. Interestingly, Stat3-C also upregulated the redox-associated protein redox factor-1 (Ref-1) and reduced apoptosis in liver following Jo2 injection by suppressing oxidative stress and redox-sensitive caspase-3 activity. These findings indicate that Stat3 activation protects against Fas-mediated liver injury by inhibiting caspase activities in redox-dependent and -independent mechanisms.

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Introduction

Signal transducer and activator of transcription-3 (Stat3) is ubiquitously expressed and is transiently activated by a large number of different ligands, including IL-6, leukemia-inhibitory factor, EGF, and PDGF, as well as by a number of oncogenic receptor- and nonreceptor (src-like) tyrosine kinases (1–5). Stat3 was first described as a molecule with DNA-binding activity, found in IL-6–stimulated hepatocytes, that was capable of selectively interacting with an enhancer element in the promoter of acute-phase genes known as the acute-phase response element (6, 7). Stat3 is activated by the entire family of IL-6–type cytokines, which signal through gp130 and related receptors, and by some growth factors, oncoproteins, and interferons (8, 9). The physiological functions of Stat3 have been extensively studied, and it is known to play crucial roles in the development of various organs and in cell proliferation (5). Though targeted disruption of the *Stat3* gene actually leads to early embryonic lethality (10), studies have been done using mice with conditional knockout (liver, lymphocyte) of Stat3 (10, 11). In liver-specific Stat3-knockout mice (LS3-KO mice), no abnormality in liver development and structure was observed, but these mice showed less mitogenic response following partial hepatectomy (11). Also, many other studies have been reported indicating that Stat3 is involved in several important immediate early response genes following partial hepatectomy (3, 12).

In addition to promoting proliferation, Stat3 also has antiapoptotic properties. When stimulated with serum withdrawal and UV irradiation, Stat3 competes with the proapoptotic effect of Stat1 in fibroblasts by inducing expression of Bcl-xL and Survivin (13). Stat3 may cause cellular proliferation by preventing apoptosis in some types of cells (13–15). However, the precise mechanism for the antiapoptotic property of Stat3 is not well known.

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Nonstandard abbreviations used: signal transducer and activator of transcription-3 (Stat3); liver-specific Stat3-knockout mice (LS3-KO mice); FLICE inhibitor protein (FLIP); constitutively activated Stat3 (Stat3-C); redox factor-1 (Ref-1); a replication-deficient adenoviral vector encoding Stat3-C (AxCAS3-C); *N*-acetyl-L-cysteine (NAC); manganese superoxide dismutase (MnSOD); reactive oxygen species (ROS); physiological saline (PS); glutamic-oxaloacetic transaminase (GOT); glutamic-pyruvic transaminase (GPT).