Amino Acids (2008) 34: 91–102
DOI 10.1007/s00726-007-0576-2
Printed in The Netherlands

Amino Acids

Expression profiles of the genes associated with metabolism and transport of amino acids and their derivatives in rat liver regeneration

C. S. Xu[1] and **C. F. Chang**[2]

[1] College of Life Science, Henan Normal University, Xinxiang, China
[2] Key Laboratory for Cell Differentiation Regulation, Xinxiang, China

Received June 5, 2007
Accepted June 21, 2007
Published online August 24, 2007;

Summary. Amino acids (AA) are components of protein and precursors of many important biological molecules. To address effects of the genes associated with metabolism and transport of AA and their derivatives during rat liver regeneration (LR), we firstly obtained the above genes by collecting databases data and retrieving related thesis, and then analyzed their expression profiles during LR using Rat Genome 230 2.0 array. The LR-associated genes were identified by comparing the gene expression difference between partial hepatectomy (PH) and sham-operation (SO) rat livers. It was approved that 134 genes associated with metabolism of AA and their derivatives and 26 genes involved in transport of them were LR-associated. The initially and totally expressing number of these genes occurring in initial phase of LR (0.5–4 h after PH), G0/G1 (4–6 h after PH), cell proliferation (6–66 h after PH), cell differentiation and structure-function reconstruction of liver tissue (72–168 h after PH) were respectively 76, 17, 79, 5 and 162, 89, 564, 195, illustrating that these LR-associated genes were initially expressed mainly in initial stage, and functioned in different phases. Frequencies of up-regulation and down-regulation of them being separately 564 and 357 demonstrated that genes up-regulated outnumbered those down-regulated. Categorization of their expression patterns into 22 types implied the diversity of cell physiological and biochemical activities. According to expression changes and patterns of the above-mentioned genes in LR, it was presumed that histidine biosynthesis in the metaphase and anaphase, valine metabolism in the anaphase, and metabolism of glutamate, glutamine, asparate, asparagine, methionine, alanine, leucine and aromatic amino acid almost were enhanced in the whole LR; as for amino acid derivatives, transport of neutral amino acids, urea, γ-aminobutyric acid, betaine and taurine, metabolism of dopamine, heme, S-adenosylmethionine, thyroxine, and biosynthesis of hydroxyproline, nitric oxide, orinithine, polyamine, carnitine, selenocysteine were augmented during the entire liver restoration. Above results showed that metabolism and transport of AA and their derivates were necessary in liver regeneration.

Keywords: Partial hepatectomy – Rat genome 230 2.0 array – Metabolism and transport of amino acids and their derivatives – Genes associated with liver regeneration

Abbreviations: AA, amino acids; GABA, γ-aminobutyric acid; IVT, in vitro transcription; LR, liver regeneration; NO, nitric oxide; PH, partial hepatectomy; PRPP, 5-phosphorylribose 1-pyrophosphate; SAM, S-adenosyl-L-methionine; SO, sham operation. Gene abbreviations are listed in Table 1.

1. Introduction

Liver has a very strong regenerative capacity (Taub, 2004) such that, after partial hepatectomy (PH), the remaining liver rapidly replicate to restore normal hepatic mass, which is called liver regeneration (LR) (Higgins and Anderson, 1931; Fausto et al., 2006). Usually, the rat liver regenerative process divided into four phases including initiation phase (0.5–4 h after PH), G0/G1 transition (4–6 h after PH), cell proliferation (6–66 h after PH), cell redifferentiation and structure-function reconstruction of liver tissue (72–168 h after PH), involves many physiological and biochemical events including cellular activation, dedifferentiation, proliferation and its regulation, redifferentiation (Fausto, 2000; Kountouras et al., 2001; Xu et al., 2004), presumably that metabolism and transport of amino acids (AA) and their derivatives are necessary in the above process. Proteins are the embodiment of life. A protein is built up from a long-chain polymer of AA, called a polyamino acid or polypeptide chain. As pointed out by Chou (2000), ''Polymer chain per se can be pretty dull. However, polypeptide chains formed by 20 different AA are more versatile because of the great number of different side chains that may be present''. These kinds of versatile architectures have made proteins possess many different features, such as assuming various three-dimensional structures (see, e.g., Chou and Zhang, 1995; Chou, 2004a) with different cleavage sites (see, e.g.,

Poorman et al., 1991; Chou, 1993, 1996; Elhammer et al., 1993) and locating at different subcellular locations (see, e.g., Glory and Murphy, 2007; Chou and Shen, 2007a; Chou and Shen, 2007b; Shen and Chou, 2007) with varieties of functions (see, e.g., Chou, 2005). Even the short signal peptide formed by AA can function as a 'zip code' directing a nascent protein to the proper location (Blobel and Dobberstein, 1975; Blobel, 1976; Chou and Shen, 2007c). Because AA are the most fundamental components and basic units of proteins, a lot of important information for drug discovery can be acquired by amino acid substitution, the so-called mutagenesis study (Chou, 2004b; Lubec et al., 2005). It is well known that the major site of nitrogen metabolism is liver where the potentially toxic nitrogen of AA is eliminated via transaminations, deamination and urea formation in liver. Previous studies have showed that glutamate synthesized from α-ketoglutarate by transamination catalyzed by GLUD1, GLS, GOT1, and GPT1, etc. (Haussinger et al., 1983), serves as a common intracellular amino donor for transamination reactions, for example, glutamine is synthesized from glutamate by the direct incorporation of ammonia under the catalysis of CORO2A and GLULD1 etc. (Zaphiropoulos and Toftgard, 1996); arginine is produced from glutamate via a series of reaction with carbamyl phosphate and aspartate catalyzed by CPS1, OAT, and OTC (Markova et al., 2005); gamma-glutamylcysteine composed of glutamate and cysteine also combines with glycine to form glutathione catalyzed by GGT1 (Djavaheri et al., 2002); in addition, glutamate is the precursor of proline and the conversion of glutamate to proline is catalyzed by PYCR2, PYCS, and SIRT7, etc. (Deuschle et al., 2004), it is believed that the fates of arginine include the production of creatine by methylation of guanidoacetate formed from arginine and glycine using S-adenosyl-L-methionine (SAM) as the methyl donor under the catalysis of enzymes including GAMT (Komoto et al., 2004) and its degradation into ornithine catalyzed by OAT and ARG2, then phosphorylation of the former metabolite is catalyzed by CKB and CKMT2 to form phosphocreatine (Shen et al., 2002) and the later is catalyzed by AGMAT, OAZ2, OAZ3, OAZIN, and ODC1 to converted into polyamines (Higaki et al., 1999; Nissim et al., 2005). Besides that, arginine is also converted into nitric oxide (NO) and citrulline depending on the action of NOS3, NOSIP, and NOSTRIN, etc. (Icking et al., 2005). About aspartate metabolism, it is formed from oxaloacetate in a reversible transamintion reaction catalyzed by GOT1, subsequently, ASNS catalyzes the production of asparagine from aspartate and glutamine (Mo et al., 2003); the studies on utilization of aspartate have demonstrated that threonine is derived from aspartate in a multibranched biosynthetic pathway involving THNSL1 (Hoeven and Steffens, 2000), that lysine synthesis is dependent on transamintion reaction between aspartate and pyruvic acid catalyzed by RGD1310111 and RGD1310475, that aspartate, cysteine and N5-methyltetrahydrofolate are utilized in methionine synthesis under the catalysis of BHMT, MSRA, MTHFD1, MTHFR, and MTRR. The ultimate end-product of lysine catabolism is acetoacetyl-CoA via a series of reactions catalyzed by AADAT, AASS, BCKDHA, BCKDHB, ECH1, ECHDC3, GCDH, and HMGCL; methionine is converted to SAM by GNMT, MAT1A, and MAT2A, and the later may be ultimately catabolized via a complex pathway to succinyl-CoA which is Krebs Cycle intermediate succinyl-CoA by CBS, CTH, BCKDHA, and BCKDHB. During the process of alanine family AA metabolism, alanine, the direct product of pyruvate transamination via GPT1 (Nishigaki et al., 1978), can be synthesized into leucine and valine by BCAT2. Leucine and valine are respectively catabolized to acetoacetate and succinyl-CoA, respectively by ACADM, ACADSB, AUH, HMGCL, and IVD and by ALDH6A1, ECH1, HADHA, HADHB, HIBADH, and HIBCH, which can provide carbon backbone for citric acid cycle. For serine family AA metabolism, 3-phosphopyruvate, converted from 3-phosphoglycerate, is suitable for subsequent transamination with glutamate to produce 3-phosphoserine, which is converted to serine by CTBP2, GRHPR, PHGDH, and PSAT1, the resulting serine may be converted into glycine by SHMT, PHGDH, and SARDH, and heme can be synthesized from glycine and succinyl-CoA by CPOX, FECH, HMBS, NFE2L1, PPOX, and UROS, etc.; while the resulting cysteine either biosynthesized from serine and hydrogen sulfide by CBS, CTH, and ALAS2 can be further converted into selenocysteine by SEPHS2. About aromatic AA metabolism, both tryptophan and phenylalanine can be synthesized from phosphoenolpyruvate with erythrose-4-phosphate separately by APRT and PCK1 (Zeitouni et al., 2002) and by PCK1 and SDHA, and the latter is hydroxylated into tyrosine by PAH. One significant fate of tyrosine is first hydroxylation and decarboxylation to dopamine by TH, DDC, and CHRNA, and then conversion to melanin, norepinephrine and epinephrine by DDT, TYRP1, GATA3; its other fate is conversion to alkapton by PNMT, TAT, HGD, and GSTZ1, and ultimately degradation to acetyl-CoA. Tryptophan, another aromatic amino acid, is either metabolite to indoleacetic acid by IND or degradation to kynurenine by AFMID, KYNU, and TDO2 or conversion to serotonin by DDC (Ishida et al., 2002). As regards histidine, its synthesis derives from 5-

phosphorylribose 1-pyrophosphate (PRPP) and glutamate by PRPS2 and MTHFD1, which is decarboxylated by HDC, yielding histamine (Joseph et al., 1990), and the later can be catabolized to α-ketoglutarate by FTCD and HAL. To investigate these biochemical events in rat liver regeneration at mRNA level, we employed the Rat Genome 230 2.0 array (Yue et al., 2001; Collins, 2006) including 274 genes associated with metabolism and transport of AA and their derivatives to check genes expression profiles in regenerating liver after 2/3 hepatectomy, and found that a total of 160 genes is LR-associated. Their expression dynamics, patterns and actions during rat hepatic repopulation were further analyzed.

2. Materials and methods

2.1 Regenerating liver preparation

264 healthy Sprague-Dawley rats (200–250 g) were obtained from the Animal Center of Henan Normal University and were randomly divided into 22 partial hepatectomy (PH) groups and 22 sham operation (SO) groups. Each group included 6 rats. The rats in PH groups were subjected to an operation removing left lateral and median lobes of their liver, as described by Higgins and Anderson (1931). The rats were killed by cervical dislocation in 0.5, 1, 2, 4, 6, 8, 12, 16, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 96, 120, 144 and 168 h post PHx, respectively, and their livers were instantly removed. The procured livers were immediately washed three times in a cold washing buffer (0.01 mol/L PBS). For each rat, about 100–200 mg liver tissues were pooled from the middle parts of right lobe while on ice. Liver tissues of six rats for each group (total liver mass: 0.1–0.2 g × 6 = 1–2 g) were gathered and mixed, and then stored at −80 °C until use. The SO group was subjected to the same procedure as the PH group but without liver removal. Control of both groups is normal rat liver. In above experiments, the laws of animal protection of China were enforced strictly.

2.2 RNA isolation and purification

Total RNA was isolated from the frozen livers according to the manual of Trizol reagent (Invitrogen Corporation, Carlsbad, California, USA) (Knepp et al., 2003) and then purified following the RNeasy mini protocol (Qiagen Inc, Valencia, CA, USA) (Nuyts et al., 2001). RNA quantity and quality were checked by gel electrophoresis, and the 260/280 nm ratio was between 1.8–2.2 (Arkin et al., 1998). Only non-degraded RNAs without DNA contamination were utilized.

2.3 cDNA, cRNA synthesis and purification

Five microgram of total RNA were converted to the first strand of cDNA by using SuperScript II RT (Invitrogen Corporation, Carlsbad, CA), and gel-purified T7-oligo dT(24) (W.M. Keck Foundation, New Haven, CT) as the primer. Second strand synthesis was performed using *E. coli* Polymerase I, DNA ligase, *E. coli* RNA H and T4 DNA Polymerase according to the manufacturer's instructions and reagents in the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen). The resulting cDNA was purified by extraction with (25:24:1) phenol: chloroform: isoamyl alcohol (Ambion Inc., Austin, TX), separation of the aqueous phase using phase lock gels and precipitation in ethanol. The purified cDNA subsequently served as template for production of biotin labeled cRNA transcript using the BioArray High Yield RNA Transcript Labeling Kit (ENZO Biochemical, New York, NY) and biotin-labeled UTP and CTP. Labeled cRNA was isolated using the RNeasy Mini Kit columns (Qiagen, Valencia, CA), quantified for purity, concentration and yield (Leong et al., 2006).

2.4 cRNA fragmentation and microarray detection

Fifteen microlitre (1 μg/μl) cRNA incubated with 5 × fragmentation buffer for 35 min at 94 °C was digested into 35–200 bp fragments, suitable for hybridization. Fragmented cRNA was hybridized to Affymetrix Rat Genome 230 2.0 Arrays (60 rpm, 16 h, 45 °C) in a rotating chamber. Arrays were washed and stained by GeneChip fluidics station 450 (Affymetrix Inc., Santa Clara, CA, USA), and scanned with a GeneChip Scanner 3000 (Affymetrix Inc., Santa Clara, CA, USA). Image processing and probe set level data analysis were performed with Affymetrix GCOS 1.2 software (Buettner et al., 2006).

2.5 Microarray data analysis

Each gene on the Rat Genome 230 2.0 Arrays is represented by 20 different 25-base cDNA oligonucleotides complementary to a cRNA target transcript (perfect match) together with specificity control oligonucleotides containing a single base substitution (mismatch) for each perfect-match. The combination of perfect-match and mismatch cDNA oligonucleotides for each gene is termed a probe set. Affymetrix-defined absolute mathematical algorithms describing perfect-match and mismatch hybridization intensities were used to define each gene as "present" or "absent" and assign a value. Binding intensity values were scaled to evaluate differential expression in rat liver treated with PH/SO. Affymetrix-defined comparison mathematical algorithms determined whether a transcript was classified as "increased", "decreased", "marginally increased", "marginally decreased" or "not changed" and a fold change in expression was calculated. Only genes that had signal present were selected from the normalized data for further analysis. This was accomplished using GeneSpring version 7.0 (Silicon Genetics, Redwood City, CA) importing data from Affymetrix MicroArray Suite (MAS 5.0) into GeneSpring as tab delimited text files (Eisen et al., 1998; Werner, 2001; Collins, 2006). Additional criteria/filters were used to classify a gene as significantly up- or down-regulated: (i) expression of a gene must be classified as increased or marginally increased (or decreased or marginally decreased) in each replicate compared to the control. (ii) The mean fold change for PH-treated gene expression must be greater than 2 compared to the control. To minimize the technical error from the microarray analysis, each sample was measured three times. The average value of three measurement results was calculated as the sum of the fold change in gene expression for PH-treated regenerative livers at each time point compared to control (Leong et al., 2006).

2.6 Rat liver regeneration-associated gene identification

Firstly, the nomenclatures of biosynthesis, catabolism and transport of each amino acid and its derivatives were adopted from the GENEONTOLOGY database (www.geneontology.org), and were input into the databases at NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu) to find out the rat, mouse and human genes associated with metabolism and transport of AA and their derivatives. In addition, according to maps of biological pathways embodied by GENMAPP (www.genmapp.org), KEGG (www.genome.jp/kegg/pathway.html) and BIOCARTA (www.biocarta.com/genes/index.asp), the genes associated with above processes were collated, and reconfirmed through literature searches of the pertinent articles. Besides the rat genes, the genes which were now known only exist in mouse and/or human and which exhibited a greater than two-fold change in the rat regenerating liver were referred to as rat homologous genes (Frago et al., 1998). The genes that displayed reproducible results with the three independent analyses and that showed a greater than two-fold change in expression in at least one time point as a significant difference

Table 1. Expression abundance of 160 genes associated with amino acids and their derivatives metabolism and transport during rat liver regeneration

Name	Gene abbr.	Associated to action	Fold difference	Name	Gene abbr.	Associated to action	Fold difference
Branched chain aminotransferase 2, mitochondrial	*Bcat2*	1[a], 1[b], 2[a], 2[b], 31[b]	3.0	D-dopachrome tautomerase	*Ddt*	6[a]	4.4
				Tyrosinase-related protein 1	*Tyrp1*	6[a]	0.4
Aldehyde dehydrogenase family 6, subfamily A1	*Aldh6a1*	1[b]	2.9, 0.4	Cholinergic receptor, nicotinic, alpha polypeptide 7	*Chrna7*	7[a]	2.5, 0.2
Hydroxyacyl-coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase/ enoyl-coenzyme A hydratase (trifunctional protein), alpha subunit	*Hadha*	1[b]	0.5	Monoamine oxidase B	*Maob*	7[b]	2.2, 0.2
				Nuclear receptor subfamily 4, group A, member 2	*Nr4a2*	7[d]	7.1, 0.4
Hydroxyacyl-coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase/ enoyl-coenzyme A hydratase (trifunctional protein), beta subunit	*Hadhb*	1[b]	2.9	GATA binding protein 3	*Gata3*	8[a]	0.4
				Solute carrier family 6, member 2	*Slc6a2*	8[c]	2.9, 0.5
				Deiodinase, iodothyronine, type I	*Dio1*	9[a]	0.3
				Peroxisome proliferator activated receptor binding protein	*Pparbp*	9[a]	5.0
3-Hydroxyisobutyrate dehydrogenase	*Hibadh*	1[b]	0.5				
3-Hydroxyisobutyryl-coenzyme A hydrolase	*Hibch*	1[b]	0.4				
Branched chain ketoacid dehydrogenase E1, alpha polypeptide	*Bckdha*	1[b], 27[b], 29[b]	4.2	Thyroglobulin	*Tg*	9[a]	0.4
				Transthyretin	*Ttr*	9[a]	6.9
Branched chain keto acid dehydrogenase E1, beta polypeptide	*Bckdhb*	1[b], 27[b], 29[b]	4.9	Deiodinase, iodothyronine, type II	*Dio2*	9[a], 9[b]	3.0
				Deiodinase, iodothyronine, type III	*Dio3*	9[b]	24.3, 0.5
Enoyl coenzyme A hydratase 1, peroxisomal	*Ech1*	1[b], 29[b], 31[b]	0.3	Solute carrier organic anion transporter family, member 4a1	*Slco4a1*	9[c]	0.4
Solute carrier family 38, member 1	*Slc38a1*	1[c], 3[c], 23[c], 27[c], 32[c], 34[c], 36[c], 39[c], 41[c], 42[c], 44[c], 46[c]	0.1				
				Adenine phosphoribosyl transferase	*Aprt*	10[a]	2.8
				Arylformamidase	*Afmid*	10[b], 11[a]	2.7, 0.4
Acetyl-coenzyme A dehydrogenase, medium chain	*Acadm*	2[b]	6.9	Indoleamine 2,3-dioxygenase	*Indo*	10[b], 11[a]	2.3, 0.4
				Kynureninase	*Kynu*	10[b], 11[a]	0.1
Acyl-coenzyme A dehydrogenase, short/branched chain	*Acadsb*	2[b]	2.4, 0.3	Tryptophan 2,3-dioxygenase	*Tdo2*	10[b], 14[a]	2.0
				Collagen-like tail subunit of asymmetric acetylcholinesterase	*Colq*	12[b]	0.3
AU RNA binding protein/enoyl-coenzyme A hydratase	*Auh*	2[b]	2.6				
				Sarcosine dehydrogenase	*Sardh*	13[b], 42[a]	2.6
Isovaleryl coenzyme A dehydrogenase	*Ivd*	2[b]	2.9	Dimethylglycine dehydrogenase precursor	*Dmgdh*	13[b], 42[b]	2.0, 0.4
3-Hydroxy-3-methylglutaryl-coenzyme A lyase	*Hmgcl*	2[b], 29[b]	2.9	Solute carrier family 5, member 7	*Slc5a7*	13[c]	0.2
Solute carrier family 7, member 6	*Slc7a6*	2[c], 17[c], 23[c], 27[c], 29[c], 32[c], 34[c], 36[c]	8.0	Solute carrier family 6, member 4	*Slc6a4*	14[b]	0.0
				Nicotinamide N-methyltransferase	*Nnmt*	15[a]	9.4, 0.3
Phosphoenolpyruvate carboxykinase 1	*Pck1*	3[a], 10[a], 16[a]	3.2	Succinate dehydrogenase complex, subunit A, flavoprotein	*Sdha*	16[a]	2.3
Fumarylacetoacetate hydrolase	*Fah*	3[b], 16[b]	0.4				
Glutathione transferase zeta 1	*Gstz1*	3[b], 16[b]	0.4	Quinoid dihydropteridine reductase	*Qdpr*	16[b]	2.0, 0.4
Homogentisate 1,2-dioxygenase	*Hgd*	3[b], 16[b]	2.5	Ornithine transcarbamylase	*Otc*	17[a]	2.0, 0.4
Tyrosine aminotransferase	*Tat*	3[b], 16[b]	2.8	Ornithine aminotransferase	*Oat*	17[a], 17[b], 22[a]	5.2
Phenylethanolamine-N-methyltransferase	*Pnmt*	4[a], 5[a]	0.1	Carbamoyl-phosphate synthetase 1, mitochondrial	*Cps1*	17[a], 36[e]	2.3
Tyrosine hydroxylase	*Th*	4[a], 5[a], 7[a]	0.4				
Activating transcription factor 2	*Atf2*	5[a]	2.6	ADP-ribosyltransferase 4	*Art4*	17[b]	6.5
Histidine decarboxylase	*Hdc*	5[a], 32[b], 33[a]	0.2	Dimethylarginine dimethylaminohydrolase 2	*Ddah2*	17[b]	2.1
Dopa decarboxylase	*Ddc*	5[a], 7[a], 14[a]	2.3, 0.5	Arginase 2	*Arg2*	17[b], 22[a]	3.6
Sulfotransferase family 1A, phenol-preferring, member 1	*Sult1a1*	5[b]	2.2, 0.2	Solute carrier family 14, member 1	*Slc14a1*	17[c]	4.7
				Solute carrier family 14, member 2	*Slc14a2*	17[c]	0.4

Solute carrier family 7, member 3	*Slc7a3*	17[c], 29[c]	2.1, 0.3
Solute carrier family 7, member 1	*Slc7a1*	17[c], 29[c], 32[c]	0.4
Nitric oxide synthase 3, endothelial cell	*Nos3**	18[a]	2.1, 0.3
Nitric oxide synthase interacting protein	*Nosip*	18[a]	10.6, 0.4
Nitric oxide synthase trafficking	*Nostrin*	18[a]	8.0
Guanidinoacetate methyltransferase	*Gamt*	19[a]	0.5
Creatine kinase, brain	*Ckb*	20[a]	2.4, 0.2
Creatine kinase, mitochondrial 2, sarcomeric	*Ckmt2*	20[a]	3.0, 0.2
Agmatine ureohydrolase	*Agmat*	21[a]	2.1, 0.4
Ornithine decarboxylase antizyme 2	*Oaz2*	21[a]	2.1
Ornithine decarboxylase antizyme 3	*Oaz3*	21[a]	2.8, 0.3
Ornithine decarboxylase antizyme inhibitor	*Oazin*	21[a]	2.3
Ornithine decarboxylase 1	*Odc1**	21[a]	3.3
Polyamine oxidase	*Paox*	21[b]	3.3
CTL target antigen	*Cth*	23[a], 25[b], 27[b]	0.0
Cystathionine beta synthase	*Cbs*	23[a], 25[b], 27[b], 41[b]	2.7
Cystinosis, nephropathic	*Ctns*	23[c]	0.3
Solute carrier family 3, member 1	*Slc3a1*	23[c]	2.4
Solute carrier family 38, member 2	*Slc38a2**	23[c], 27[c], 32[c], 34[c], 36[c], 39[c], 41[c], 42[c], 44[c]	10.6, 0.5
Solute carrier family 1, member 4	*Slc1a4*	23[c], 34[c], 41[c]	13.3
Solute carrier family 7, member 10	*Slc7a10*	23[c], 34[c], 41[c], 42[c], 46[c]	7.5
Glutamate-cysteine ligase, catalytic subunit	*Gclc*	24[a]	2.1, 0.5
Glutathione synthetase	*Gss*	24[a]	2.3
Gamma-glutamyltransferase 1	*Ggt1*	24[b], 39[b]	3.4, 0.2
Betaine-homocysteine methyltransferase	*Bhmt*	25[b], 27[a]	5.0
Selenophosphate synthetase 2	*Sephs2*	26[a]	3.5
Selenocysteine lyase	*Scly*	26[b]	0.1
Methionine sulfoxide reductase A	*Msra*	27[a]	0.5
5,10-methylenetetrahydrofolate reductase	*Mthfr*	27[a]	3.7, 0.4
5-Methyltetrahydrofolate-homocysteine methyltransferase reductase	*Mtrr*	27[a]	2.8, 0.3
Methylenetetrahydrofolate dehydrogenase	*Mthfd1*	27[a], 32[a], 32[b]	2.1
Methionine adenosyltransferase I, alpha	*Mat1a**	27[b], 28[a]	6.0
Methionine adenosyltransferase II, alpha	*Mat2a**	27[b], 28[a]	3.8
Glycine N-methyltransferase	*Gnmt*	28[a]	2.5, 0.4
Similar to RIKEN cDNA 0610012D14	*RGD1310111*	29[a]	0.3
Similar to RIKEN cDNA 0610010D20	*RGD1310475*	29[a]	0.3
Glutamate oxaloacetate transaminase 1	*Got1*	29[a], 37[a], 45[a], 45[b]	12.4
Aminoadipate aminotransferase	*Aadat*	29[b]	0.4
Aminoadipate-semialdehyde synthase	*Aass*	29[b]	3.8
Glutaryl-coenzyme A dehydrogenase	*Gcdh*	29[b]	5.3, 0.3
Enoyl coenzyme A hydratase domain containing 3	*Echdc3*	29[b], 31[b]	0.3
Butyrobetaine (gamma), 2-oxoglutarate dioxygenase 1	*Bbox1*	30[a]	6.0, 0.4
Trimethyllysine hydroxylase, epsilon	*Tmlhe*	30[a]	3.0, 0.4
Threonine synthase-like 1	*Thnsl1*	31[a], 46[a]	0.2
Phosphoribosyl pyrophosphate synthetase 2	*Prps2*	32[a]	3.1
Formiminotransferase cyclodeaminase	*Ftcd*	32[b]	0.2
Histidine ammonia lyase	*Hal*	32[b]	1.3, 0.3
Solute carrier family 38, member 5	*Slc38a5**	32[c], 36[c], 41[c], 42[c], 44[c]	3.0
Solute carrier family 38, member 3	*Slc38a3**	32[c], 36[c], 44[c]	5.3
Proteoglycan 3	*Prg3*	33[a]	0.2
Glutamic pyruvic transaminase 1, soluble	*Gpt1*	34[a], 34[b], 37[a]	3.5, 0.5
Ureidopropionase, beta	*Upb1*	35[a]	0.4
Coronin, actin binding protein 2A	*Coro2a*	36[a]	2.5, 0.3
Glutamate-ammonia ligase domain containing 1	*Gluld1*	36[a]	4.3
Phosphoribosylformylglycinamidine synthase	*Pfas*	36[b]	0.3
Glutaminase	*Gls*	36[b], 37[a]	2.4
Gamma-glutamyl hydrolase	*Ggh*	36[b], 37[b]	2.1
Carbamyl phosphatate synthetase 2	*Cad*	36[e]	2.8, 0.3
Glutamine fructose-6-phosphate transaminase 1	*Gfpt1*	36[e]	0.5
Glutamine-fructose-6-phosphate transaminase 2	*Gfpt2*	36[e]	0.5
LEM domain containing 1	*Lemd1*	36[e]	2.6, 0.3
Proline dehydrogenase 1	*Prodh*	37[a]	0.4
Proline dehydrogenase 2	*Prodh2*	37[a]	0.3
Aldehyde dehydrogenase family 5, subfamily A1	*Aldh5a1*	37[b], 38[b]	5.4
Solute carrier family 17, member 6	*Slc17a6*	37[c]	2.6, 0.1
4-Aminobutyrate aminotransferase	*Abat*	38[b]	2.1
Solute carrier family 6, member 1	*Slc6a1*	38[c]	6.5, 0.3
Solute carrier family 6, member 11	*Slc6a11*	38[c]	6.0
Solute carrier family 6, member 12	*Slc6a12*	38[c]	4.0, 0.4
Pyrroline-5-carboxylate reductase family, member 2	*Pycr2*	39[a]	3.0, 0.2
Pyrroline-5-carboxylate synthetase	*Pycs*	39[a]	0.4
Sirtuin 7	*Sirt7*	39[a]	9.8, 0.3
Solute carrier family 6, member 7	*Slc6a7*	39[c]	3.1
Procollagen-proline, 2-oxoglutarate 4-dioxygenase, alpha II polypeptide	*P4ha2*	40[a]	7.2
3-Phosphoglycerate dehydrogenase	*Phgdh*	40[a], 42[a]	6.5, 0.3
C-terminal binding protein 2	*Ctbp2*	41[a]	0.5
Glyoxylate reductase/hydroxypyruvate reductase	*Grhpr*	41[a]	0.4
Phosphoserine aminotransferase 1	*Psat1*	41[a]	0.4
Serine dehydratase	*Sds*	41[b]	14.5, 0.5
Solute carrier family 6, member 5	*Slc6a5*	42[c]	0.3
Solute carrier family 6, member 9	*Slc6a9*	42[c]	0.4
Aminolevulinic acid synthase 2	*Alas2*	43[a]	2.1

(*continued*)

Table 1 (*continued*)

Name	Gene abbr.	Associated to action	Fold difference	Name	Gene abbr.	Associated to action	Fold difference
Coproporphyrinogen oxidase	*Cpox*	43[a]	11.0, 0.5	Biliverdin reductase A	*Blvra*	45[a]	3.5
Hydroxymethylbilane synthase	*Hmbs*	43[a]	0.5	Aspartoacylase	*Aspa*	45[a], 45[b]	4.3
Nuclear factor, erythroid derived 2,-like 1	*Nfe2l1*	43[a]	2.9	Similar to Hypothetical protein MGC59076	*RGD1309144*	46[a]	0.5
Protoporphyrinogen oxidase	*Ppox*	43[a]	0.1	Acyl-CoA synthetase long-chain family member 1	*Acsl1*	46[b]	2.4, 0.4
Uroporphyrinogen III synthase	*Uros*	43[a]	3.7	Acyl-CoA synthetase long-chain family member 4	*Acsl4*	46[b]	2.1, 0.4
Alpha 1 microglobulin/bikunin	*Ambp*	43[b]	5.1	Acyl-CoA synthetase long-chain family member 6	*Acsl6*	46[b]	2.6
Similar to HCV NS3-transactivated protein 1	*RGD1306332*	44[a]	0.3	Solute carrier family 18, member 1	*Slc18a1*	47[c]	7.8, 0.4
Ferrochelatase	*Fech*	44[c]	2.2	Solute carrier family 6, member 6	*Slc6a6**	48[c]	2.7, 0.3

* Reported genes associated with liver regeneration; [a] biosynthesis; [b] catabolism; [c] transport; [d] regulation; [e] other. *1* Valine; *2* Leucine; *3* Tyrosine; *4* Epinephrine; *5* Catecholamine; *6* Melanin; *7* Dopamine; *8* Norepinephrine; *9* Thyroid hormone; *10* Tryptophan; *11* Kynurenine; *12* Acetylcholine; *13* Choline; *14* Serotonin; *15* Nicotinamide; *16* Phenylalanine; *17* Arginine; *18* Nitrous oxide; *19* Creatine; *20* Phosphocreatine; *21* Polyamine; *22* Ornithine *23* Cysteine; *24* Glutathione; *25* Homocysteine; *26* Selenocysteine; *27* Methionine; *28* S-adenosylmethionine; *29* Lysine; *30* Carnitine; *31* Isoleucine; *32* Histidine; *33* Histamine; *34* Alanine; *35* Beta-alanine; *36* Glutamine; *37* Glutamate; *38* Gamma-aminobutyric acid; *39* Proline; *40* 4-hydroxyproline; *41* Serine; *42* Glycine; *43* Heme; *44* Asparagine; *45* Aspartate; *46* Threonine; *47* Monoamine; *48* Taurine

($P \leq 0.05$) or an extremely significant difference ($P \leq 0.01$) between partial hepatectomy (PH) and sham operation (SO) by *F*-test, were included as being associated with rat liver regeneration.

3. Results

3.1 The general description of expression of genes associated with metabolism and transport of amino acids and their derivatives during rat liver regeneration

According to the information from databases at NCBI, GENMAPP, KEGG, BIOCARTA, and RGD, etc., 324 and 51 genes are respectively involved in metabolism and transport of amino acids (AA) and their derivatives. Subsequently, according to Rat Genome 230 2.0 array, 226 and 48 of the above genes were contained in the chip. Among them, 134 and 26 genes revealed meaningful expression changes in at least one time point post partial hepatectomy (PH), showed significant or extremely significant differences in expression when comparing partial hepatectomy with sham operation (SO), and had reproducible results with three independent analysis by Rat Genome 230 2.0 array, suggesting that they were associated with liver regeneration (LR). Chip analysis showed that in the metabolism and transport mentioned above, correspondingly, 54 and 9 genes exhibited a greater than twofold increase in the expression level, and another 40 and 9 genes were reduced more than twofold, whereas the rest of the total regulated genes (respectively 40 and 8) displayed dynamically up- and down-regulated pattern during LR. Fold change in up-regulated expression and down-regulated expression ranged respectively from 2-fold to 24-fold and from 2-fold to 100-fold (Table 1).

3.2 Expression changes of genes associated with metabolism and transport of amino acids and their derivatives during rat liver regeneration

At each time point of LR, the numbers of initial up-, down-regulated genes and the total times of up, down-regulated genes were in sequence: both 21 and 9 at 0.5 h; 21, 8 and 38, 14 at 1 h; 7, 4 and 29, 9 at 2 h; 4, 2 and 33, 9 at 4 h; 2, 9 and 30, 17 at 6 h; 2, 3 and 31, 14 at 8 h; 1, 11 and 27, 30 at 12 h; 13, 4 and 30, 17 at 16 h; 3, 9 and 21, 32 at 18 h; 2, 5 and 23, 26 at 24 h; 3, 1 and 20, 13 at 30 h; 0, 1 and 15, 26 at 36 h; 0, 0 and 23, 7 at 42 h; 0, 4 and 18, 25 at 48 h; 0, 1 and 19, 18 at 54 h; 0, 0 and 20, 17 at 60 h; 5, 0 and 36, 9 at 66 h; 1, 0 and 17, 11 at 72 h; 1, 0 and 32, 9 at 96 h; 1, 0 and 26, 19 at 120 h; 0, 1 and 26, 15 at 144 h; 1, 0 and 29, 11 at

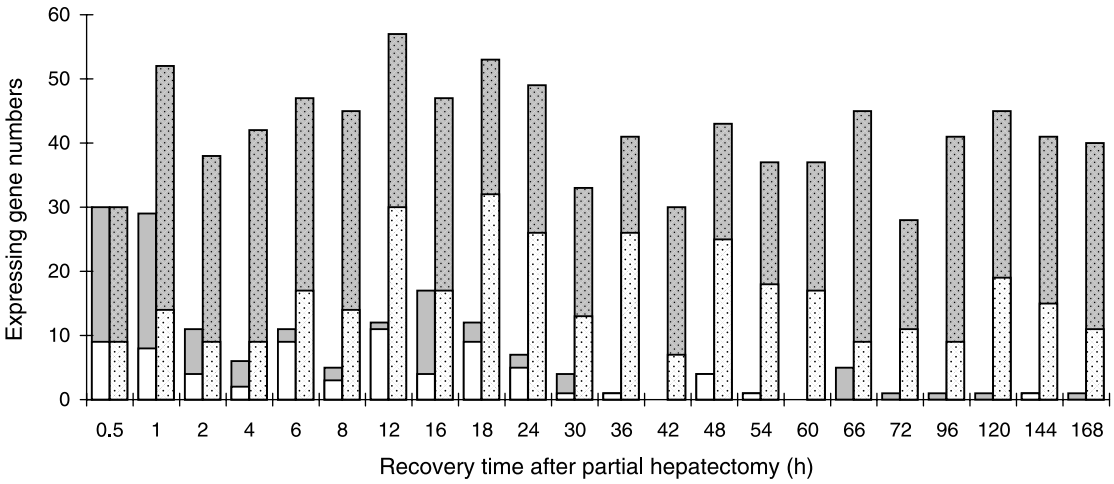

Fig. 1. The initial and total expression profiles of 160 genes associated with amino acids and their derivatives metabolism and transport at each time point of liver regeneration. Blank bars: initial expressing genes. Dotted bars: the total expressing genes; grey-background columns: up-regulated genes; white-background columns: down-regulated; gene expression changes span the entire liver regeneration. The initial up-regulation genes and initial down-regulation genes are predominant, respectively at 0.5–4, 16, 30, 66 h time points and at 6–12, 18, 24, 48 h time points, whereas very few genes are initially expressed at other time points

168 h. As a whole, 88 and 72 genes were initially up-regulated and down-regulated during LR, i.e. in initiation stage (0.5–4 h after PH), G0/G1 transition (4–6 h after PH), cell proliferation (6–66 h after PH), cell differentiation and structure-function rebuilding (72–168 h after PH), the number of initial up-regulation and down-regulation was 53 and 23, 6 and 11, 31 and 48, 4 and 1, respectively. The total frequencies of up-regulation and down-regulation of the genes during LR was 564 and 357, in parallel, was 121 and 41, 63 and 26, 313 and 251, 130 and 65 in four stages of LR.

3.3 Expression patterns of genes associated with metabolism and transport of amino acids and their derivatives during rat liver regeneration

According to gene expression dynamics, 160 LR-related genes were classified into 22 patterns (Fig. 2), including 10 patterns for 64 up-expressing genes (1–10), 9 types for 48 down-expressing genes (11–19) and 3 groups for 48 up/down-regulated genes. Among them, (1) 12 genes at one time point, i.e. 1, 6, 16, 66, 96, and 168 h after partial hepatectomy (PH); (2) 5 at two time points, i.e. 8 and 18 h, 8 and 54 h, 16 and 96 h, 30 and 42 h, 120 and 168 h; (3) 8 at three points; (4) 3 at four time points; (5) 2 at one phase, i.e. 1–8 h, 4–6 h; (6) 2 at three phases; (7) 8 at one time point/one phase, i.e. 16 and 96–120 h, 66 and 120–168 h, 72 and 120–168 h, 42 and 24–30 h, 48 and 0.5–4 h, 66 and 6–24 h, 66 and 18–24 h, 120 and 1–24 h; (8) 6 at one time point/two phases; (9) 2 at two time points/one phase; (10) 15 at multiple time points/multiple phases; (11) 13 genes at one time point, i.e. 2, 6, 12, 18, 24, 36, or 168 h; (12) 7 genes at two time points involving 0.5 and 36 h, 1 and 144 h, 12 and 36 h, 18 and 54 h, 48 and 60 h, 48 and 120 h, 54 and 120 h; (13) 1 gene at three time points; (14) 5 genes at one phase involving 6–24, 8–12, 12–18, 16–30 h; (15) 2 genes at three phases; (16) 5 genes at one time point/one phase, i.e. 12 and 0.5–4 h, 18 and 48–60 h, 36 and 6–18 h, 36 and 12–18 h, 48 and 18–30 h; (17) 4 in one time point/two phases; (18) 6 genes down at two time points/one phase; (19) 6 genes down at more time points/more phases; (20) 21 genes displayed up-expression in relative more points in time; (21) 17 genes did just the opposite, and (22) as for other 10 genes, the frequencies of up-regulation were equal to down-regulation.

4. Discussion

Amino acids (AA), as the primary components of protein, are the precursors of nucleotides, nucleotide coenzymes, heme, hormones, neurotransmitters, nitric oxide (NO), glutathione and so on (Kaliman et al., 2005; Lam et al., 2005; Pledgie et al., 2005; Soh et al., 2005; Vemuganti, 2005). All tissues have some capability for synthesis and transport of AA, and conversion of one kind of AA into another and into the corresponding amino acid derivatives. Moreover, the liver is the major site of nitrogen metabolism in the body (Lorenzi et al., 1993). As previously stated, the above biochemical processes involve

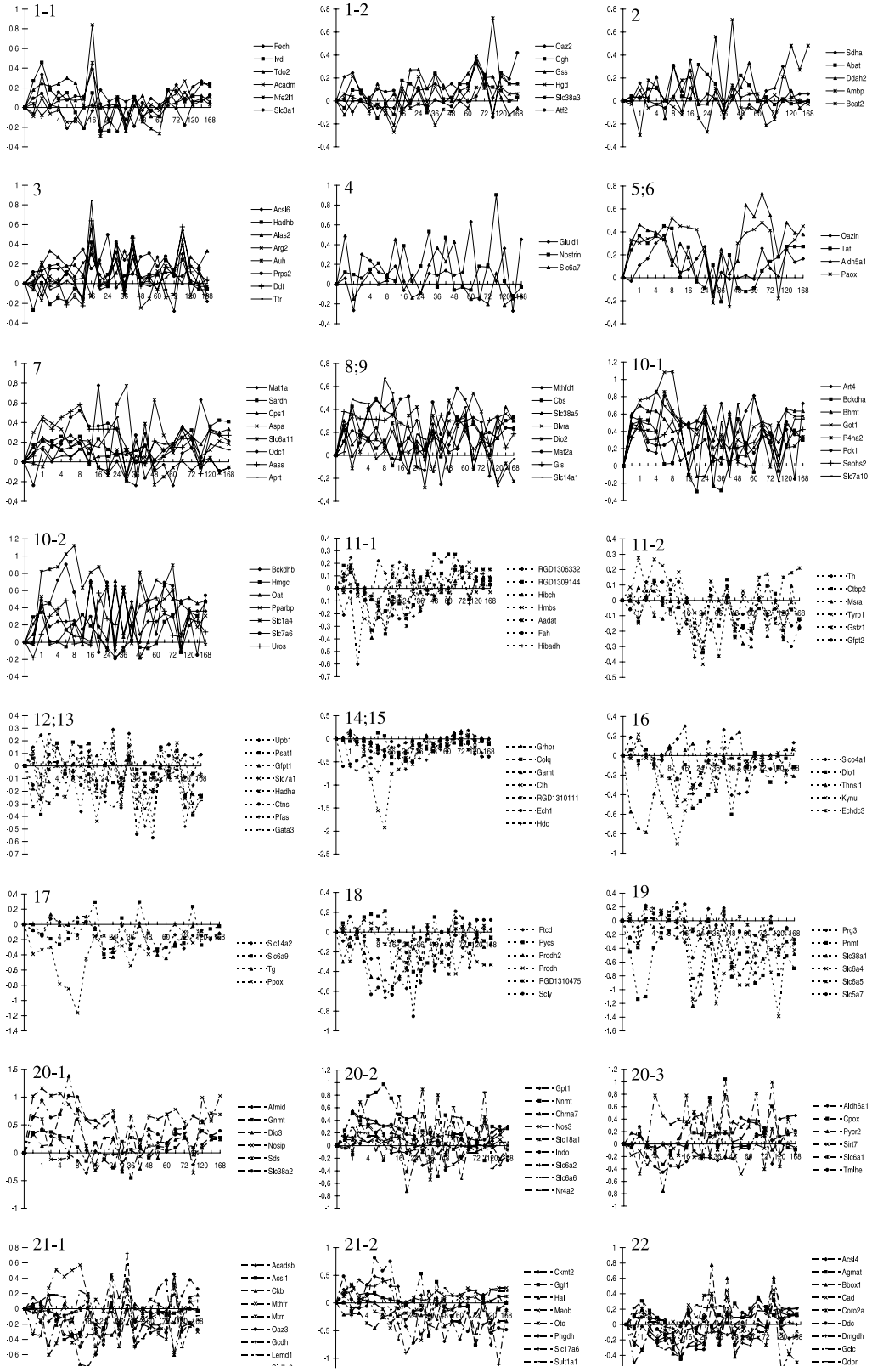

Fig. 2. Twenty-two expression patterns of 160 genes associated with amino acids and their derivatives metabolism and transport during liver regeneration. Expression patterns were obtained by analysis of detection data of Rat Genome 230 2.0 array with Microsoft Excel. 1–10: 63 up-regulated genes; 11–19: 49 down-regulated genes; 20–22: 48 up/down-regulated genes. X-axis represents recovery time after PH (h); Y-axis shows logarithm ratio of the signal values of genes at each time point to control. Real line: up-regulated genes; Broken line: down-regulated genes; real-broken line: up/down-regulated genes

numerous genes. The major goal of this paper is to identify the combined role of them in this biological event after partial hepatectomy (PH) and predict the state of metabolism and transport of AA and their derivatives in liver regeneration (LR) (Xu et al., 2005). In this article, the study on aliphatic amino acid metabolism in rat LR showed that glutamate synthesis genes *gls* and *got1*, glutamine synthesis gene *gluld1*, glutamine catabolism gene *ggh* and glutathione biosynthesis gene *ggt1* displayed up-regulated expression, suggesting that glutamate and glutamine metabolism, and glutathione biosynthesis are enhanced. The expression levels of the following genes: *cps1* and *oat* participating in conversion of arginine from glutamate, *oat* and *arg2* catalyzing arginine into ornithine, *oaz2*, *oazin* and *odc1* involved in incorporation of arginine and ornithine into polyamines, *nos3*, *nosip* and *nostrin* responsible for NO produced by arginine, are significantly increased, supposing an enhanced glutamate and its derivates formation in LR. Among the above genes, the up-regulated expression of *odc1* at 24 h after PH reported by Fukuda et al. (1999) is consistent with our result. NO biosynthesis gene *nos3* was shown to be up-regulated as reported by Minin et al. (2005) during CCl4-induced rat LR, while in this paper this gene was identified as being up-regulated at 2–4 h after PH, which maybe due to the utilization of different models of LR. It has been well known that NO can prevent the TNF-α-mediated activation of proapoptotic caspase-3 (Pahlavan et al., 2006), according to the result of the study, an increase of NO production maybe enhance the anti-apoptotic capacity of regenerating liver. Proline biosynthesis gene *pycs*, and proline catabolism genes *prodh* and *prodh2* were all down-regulated, which probably explains why proline metabolism is attenuated in LR. Hydroxyproline, one of ingredients of collagen, is converted from proline via *p4ha2* (Farhat et al., 1979), and its expression level is elevated at 0.5–30 h after PH, speculating that collagen biosynthesis is promoted in corresponding time.

Aspartate family amino acid metabolism consists of a series of chemical reactions involving in AA of aspartate family, comprising aspartate, asparagines, lysine, methionine and threonine. Among the above metabolism-involved genes, *aspa* and *got1* catalyze the conversion of aspartate into asparagine, *bhmt* and *mthfd1* catalyze methionine biosynthesis from aspartate, and condensation of the resulting methionine and ATP catalyzed by *mat1a* and *mat2a* yields S-adenosyl-L-methionine (SAM) (Sakata et al., 1993), which is catabolized into succinyl-CoA by *bckdha*, *bckdhb* and *cbs* SAM to enter citric acid cycle. The observably increased expression of the above genes may be accountable for the enhanced metabolic processes of asparate, asparagine and methionine in LR. Especially, Frago et al. (1998) reported that *mat1a* and *mat2a* were up-regulated at 4–36 h after PH, which was consistent with our result. Threonine biosynthesis-related genes *rgd1309144* and *thnsl* were significantly down-expressed, and threonine degradation-related genes *acsl1*, *acsl4* and *acsl6* were down- at prophase and up- at anaphase, maybe reflecting that threonine metabolism is inhibited at prophase of LR and enhanced at anaphase. The reduced expression levels of *rgd1310111* and *rgd1310475* involved in lysine biosynthesis from aspartate, the elevated abundance of 4 genes including *aass* in lysine degradation into acetoacetyl-CoA, and the up/down-regulation of *bbox1* (Galland et al., 2002) and *tmlhe* in conversion of lysine to carnitine in LR suggest the decrease of lysine pool in LR.

Pyruvate family amino acid metabolic process is defined as the biochemical reactions involving any amino acid that requires pyruvate for its synthesis, for example, alanine formed directly from pyruvate by *gpt1* can be converted into leucine and valine by *bcat2*, and the resultant leucine and valine will be respectively catabolized into two intermediates of citric acid cycle: acetoacetate by 5 genes including *acadm* and succinyl-CoA by 9 genes including *hadhb*, *aldh6al* and *hibch*. The above-mentioned genes showed the very complicated expression profiles, demonstrating metabolic action of alanine and leucine is intricate in LR.

During the metabolic process of serine family AA, serine, synthesized from 3-phosphoglycerate and glutamate, is transformed to glycine or cysteine, additionally, heme synthesis begins with condensation of glycine and succinyl-CoA; cysteine is converted to selenocysteine. The chip detection showed that 4 serine synthesis-related genes including *ctbp2* yielded augmented expression at prophase of LR and were reduced at metaphase and anaphase; *sardh*, catalyzing the conversion of serine into glycine, is significantly induced at metaphase, inferring an increased activity of serine biosynthesis at prophase and glycine biosynthesis at middle phase. Bilirubin, an antioxidant of potential physiological importance, is a secondary degradation product of heme catalyzed by 7 catabolism-related genes such as *alas2*, and expression of them appeared to be exceedingly complex in LR; meanwhile, heme degradation-associated genes *ambp* and *blvra* were up-regulated (Allhorn et al., 2002; Whitby et al., 2002), hypothesizing that the possible increase in the output of bilirubin in LR can improve the antioxidant capacity of

regenerating liver. The genes *cbs* and *cth* are involved in cysteine formation from serine, and their expression was significantly lower than control at prophase in LR while higher than that of control at anaphase; *sephs2*, which is responsible for formation of selenocysteine from cysteine, was up-regulated almost in the whole LR. From above results, it is presumed thats cysteine biosynthesis and conversion of cysteine to seleoncysteine are both enhanced at anaphase in LR.

2/3 hepatectomy results in the changeable expression of many genes encoding proteins participating in aromatic amino acid family metabolism, for example, APRT, PCK1 and SDHA, responsible for biosynthesis of aromatic amino acids from phosphoenopyavate acid and erythrose-4-phosphate, were markedly activated, suggesting that aromatic amino acids biosynthesis might be enhanced in LR; both the genes playing the role in synthesis of dopamine from tyrosine and those in synthesis of catecholamine from tyrosine show the complicated expression patterns: in dopamine biosynthesis, *th* was detected as down-regulated at least twofold, *chrna7* and *ddc* were up/down-regulated, while in catecholamine synthesis, *atf2* was up-regulated, *hdc*, *pnmt* and *th* were down-regulated, and *ddc* was up/down-regulated; *ddt* and *tyrp1* catalyze the conversion of tyrosine into melanin, and the former gene was up- and the latter was down-regulated. The intricate expression changes of above genes maybe explain the great fluctuation of the above biochemical events in LR. Thyroxine, having an important role in glycogen decomposition and nervous system development (Hernandez et al., 2006), is synthesized from tryptophan by *pparbp, dio2* and *ttr* whose expression level were increased in LR, and thyroxine catabolism-involved gene *dio3* also displays the similar trend pattern, suggesting an enhancement of thyroxine metabolism in LR. About 95% of tryptophan was previously reported to be metabolized to kynurenic acid, and only a very small portion of tryptophan is converted to serotonin and nicotinamide after liver injury. In our study, *indo* and *afmid* involved in tryptophan catabolism through kynurenine pathway, *ddc* in serotonin synthesis and *nnmt* in nicotinamide synthesis showed up/down-regulation, indicating the complex of tryptophan metabolism in LR, and their significance in LR needs to be further studied.

In histidine family amino acid metabolic process, histidine is synthesized from 5-phosphorylribose 1-pyrophosphate (PRPP) and glutamate by *prps2* and *mthfd1*, with subsequent decarboxylation by *hdc* and *prg3*, to produce histamine. The former two genes yielded augmented expression, and the latter two are lowered significantly, speculating that histidine biosynthesis is greatly changeable at metaphase and anaphase in LR.

The study carried out by Dransfeld et al. (2005) on the expression of genes related with transport of AA and their derivatives after PH showed that the expression level of *slc38a5* arose markedly at 3 and 6 h time points, which is in agreement with our results; however, that of *slc38a2*, *slc38a3*, and *slc6a6* was significantly decreased, differing from the expression changes measured in our study. The chip analysis in our research indicated that there was the increased expression abundance of *slc3a1* responsible for homocysteine transport, *slc1a4* for high-affinity short-chained-amino-acids transport, *slc6a7* for proline transport, *slc7a10* and *slc7a6* for neutral amino acids transport, *slc14a1* for arginine and urea transport, and *slc6a11* for transport of γ-aminobutyric acid (GABA), betaine and taurine during LR; the expression of the following transport-related genes: *ctns* involved in cysteine transport, *slc6a9* and *slc6a5* in glycine, *slc7a1* in cationic amino acids, *slc14a2* in arginine and urea, *slc38a1* in short-chained-neutral-amino-acids, *slc5a7* in vitamin B, *slc6a4* in serotonin, were all down-regulated during the regenerative process of rat liver; the rest of transport-related genes, such as arginine and lysine transport-related genes *slc7a3*, glutamate transport-related gene *slc17a6*, GABA transport-related gene *slc6a1*, GABA and betaine transport-related gene *slc6a12*, norepinephrine transport-related gene *slc6a2*, monoamine transport-related gene *slc18a1*, were up-expressed in some time points while down-expressed in others. On the basis of the above results, it is presumable that transport of neutral amino acid, urea, GABA, betaine and taurine is facilitated almost in the whole hepatic restoration.

Taken together, the treatment of experimental material in this study is characterized by comparatively long time and multiple time points, and high-throughput gene expression technique is used to investigate the expression changes of genes involved in metabolism and transport of AA and their derivates post rat partial hepatectomy. The analysis proves preliminarily that valine metabolism at anaphase and histidine biosynthesis at metaphase and anaphase are enhanced; there is an increase in both metabolism of glutamate, glutamine, asparate, asparagine, methionine, alanine, leucine, aromatic amino acid and transport of neutral amino acids, urea, GABA, betaine and taurine almost in the whole LR; both metabolism of dopamine, heme, SAM, thyroxine and biosynthesis of hydroxyproline, NO, orinithine, polyamine, carnitine, selenocysteine were augmented during the whole LR. However, gene → mRNA → protein → function are influ-

enced by many factors including proteins interaction. Therefore, the above results need to be further analyzed by the techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction, etc.

Acknowledgement

This work was supported by the National Basic Research 973 Pre-research Program of China (No. 2006CB708506).

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Authors' address: Prof. Dr. C. S. Xu, College of Life Science, No. 46, Jianshe RD, Henan, Xinxiang 453007, China

Fax: +86 373 3326524, E-mail: xucs@x263.net