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Research Article

Transcriptome atlas of glutamine family amino acid metabolism-related genes in eight regenerating liver cell types

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Abstract

To explore glutamine family amino acid metabolism of eight liver cell types in rat liver regeneration, eight kinds of rat regenerating liver cells were isolated by using the combination of Percoll density gradient centrifugation and immunomagnetic bead methods, then Rat Genome 230 2.0 Array was used to detect the expression profiles of the genes associated with metabolism of glutamine family amino acid in rat liver regeneration and finally how these genes involved in activities of eight regenerating liver cell types were analysed by the methods of bioinformatics and systems biology. The results showed that in the priming stage of liver regeneration, hepatic stellate cells and sinusoidal endothelial cells transformed proline and glutamine into glutamate; hepatocytes, hepatic stellate cells, sinusoidal endothelial cells and dendritic cells catabolized glutamate to 2-oxoglutarate or succinate; hepatic stellate cells and sinusoidal endothelial cells catalysed glutamate into glutamyl-tRNA for protein synthesis; urea cycle, which degraded from arginine, was enhanced in biliary epithelia cells, sinusoidal endothelial cells and dendritic cells; synthesis of polyamines from arginine was enhanced in biliary epithelia cells, sinusoidal endothelial cells, Kupffer cells and dendritic cells; the content of NO was increased in sinusoidal endothelial cells and dendritic cells; degradation of proline was enhanced in hepatocytes and biliary epithelia cells. In the progress stage, biliary epithelia cells converted glutamine into GMP and glucosamine 6-phosphate; oval cells converted glutamine into glucosamine 6-phosphate; hepatic stellate cells converted glutamine into NAD; the content of NO, which degraded from arginine, was increased in biliary epithelia cells, oval cells, pit cells and dendritic cells. In the termination stage, oval cells converted proline into glutamate; glutamate degradation, which degraded from arginine, was enhanced in hepatocytes and dendritic cells; the content of NO was increased in oval cells, sinusoidal endothelial cells, pit cells and dendritic cells. The synthesis of creatine phosphate was enhanced in hepatocytes, biliary epithelia cells, pit cells and dendritic cells in both progress and termination stages. In summary, glutamine family amino acid metabolism has some differences in liver regeneration in different liver cells.

Keywords: glutamate; glutamine; proline; rat liver regeneration; Rat Genome 230 2.0 Array

1. Introduction

The liver has unusual properties of regeneration (Yokoyama et al., 2007) and contains many cell types including HC (hepatocytes), BEC (biliary epithelial cells), HSC (hepatic stellate cells), OC (oval cells), SEC (sinusoidal endothelial cells), KC (Kupffer cells), PC (pit cells), DC (dendritic cells) and so on (Taub, 2004). After PH (partial hepatectomy), the remaining liver rapidly replicate to restore normal hepatic mass, which is called LR (liver regeneration) (Kountouras et al., 2001). Among them, the protein biosynthesis is essential. It has been well-documented that glutamate, glutamine, argine, proline are important components of proteins and 49 genes involved in their metabolism (Osband and Cashon, 1990; Ogata et al., 1999; Doniger et al., 2003; Wang et al., 2007). GLS2 (glutaminase 2), OAT (ornithine aminotransferase) and ALDH4A1 (aldehyde dehydrogenase 4 family member A1), PRODH (proline dehydrogenase) catalysed glutamine, ornithine and proline into glutamate, respectively (Crabtree and Newsholme, 1970; James et al., 1998; Cañas et al., 2008). GLUD1 (glutamate dehydrogenase 1), GOT1 (glutamic-oxaloacetic transaminase 1), GOT2 (glutamic-oxaloacetic transaminase 2), GPT (glutamic-pyruvate transaminase) or GPT2 (glutamic-pyruvate transaminase 2) catabolized glutamate to ketoglutarate, which was completely degraded through TCA (tricarboxylic acid) cycle (Zhang et al., 2004). GAD1 (glutamate decarboxylase 1), GAD2 (glutamate decarboxylase 2), ABAT (4-aminobutyrate aminotransferase) and ALDH5A1 (dehydrogenase 5 family member A1) catabolized glutamate to succinate, which was completely degraded through TCA cycle (Michelsen et al., 1991).

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Abbreviations: ALDH4A1, aldehyde dehydrogenase 4 family member A1; ALDH5A1, dehydrogenase 5 family member A1; BEC, biliary epithelial cells; CK, creatine kinase; DC, dendritic cells; GAD1, glutamate decarboxylase 1; GAD2, glutamate decarboxylase 2; GATM, glycine amidinotransferase; GFPT1 or GFPT2, glutamine-fructose-6-phosphate transaminase 1 or 2; GLUD1, glutamate dehydrogenase 1; GLUL, glutamate-ammonia ligase; GMPS, guanine monophosphate synthetase; GOT1, glutamic-oxaloacetic transaminase 1; GOT2, glutamic-oxaloacetic transaminase 2; GPT, glutamic-pyruvate transaminase; HC, hepatocytes; HSC, hepatic stellate cells; KC, Kupffer cells; LAP3, leucine aminopeptidase 3; LR, liver regeneration; NADSYN1, NAD synthetase 1; NOS, nitric oxide synthase; OC, oval cells; P4HA1, P4HA3, proline 4-hydroxylase, alpha polypeptide I or III; PC, pit cells; PH, partial hepatectomy; PRODH, proline dehydrogenase; SEC, sinusoidal endothelial cells; SRM, spermidine synthase.

Glutamyl-prolyl-tRNA synthetase (EPRS) or glutamyl-tRNA synthetase 2 (EARS2) catalysed glutamate to glutamyl-tRNA for protein synthesis; GLUL (glutamate-ammonia ligase) catalysed glutamate to glutamine (Deuel et al., 1978). Glutamine is the interorganizational carrier of nitrogen and important precursor of protein and nucleic acid. Carbamoyl-phosphate synthetase 2 (CAD) catalysed glutamine to carbamoyl amino acid, which is the precursor of pyrimidine synthesis (Mori et al., 1975); PPAT (phosphoribosyl pyrophosphate amidotransferase) catalysed glutamine to 5-phosphoribosylamine, which is the precursor of purine synthesis (Koenigsknecht et al., 2007); GFPT1 or GFPT2 (glutamine-fructose-6-phosphate transaminase 1 or 2) catalysed glutamine to glucosamine 6-phosphate, which involved into aminosugar metabolism (Huynh et al., 2000); NADSYN1 (NAD synthetase 1) catalysed glutamine to NAD (Hara et al., 2003), GMPS (guanine monophosphate synthetase) catalysed glutamine to GMP (Hirai et al., 1987), glutaminyl-tRNA synthetase (QARS) catalysed glutamine to glutaminyl-tRNA for protein synthesis. Arginine was catalysed to release urea by ARG (arginase), the balance of urea cycle is maintained by OTC (ornithine carbamoyltransferase), ASS (argininosuccinate synthetase), ASL (argininosuccinate lyase) (Tygstrup et al., 1995); arginine was catalysed by NOS (nitric oxide synthase) to release NO, which is important gaseous signal molecule (D'Souza et al., 2003; Yonekura et al., 2003; Panda et al., 2004); arginine was catalysed into creatine phosphate by GATM (glycine amidinotransferase, GAMT (guanidinoacetate *N*-methyltransferase) and CK (creatine kinase) (Wyss and Kaddurah-Daouk, 2000); arginine was catalysed into polyamines by ODC, SRM (spermidine synthase) and SMS (spermine synthase) (Marty et al., 1967; Wyss and Kaddurah-Daouk, 2000; Montañez et al., 2007); arginine was catalysed into arginyl-tRNA for protein synthesis by arginyl-tRNA synthetase (RARS). Proline may be generated from a small peptide *in vivo* by LAP3 (leucine aminopeptidase 3); P4HA1, P4HA3 (proline 4-hydroxylase, alpha polypeptide I or III), PRODH2, ALDH4A1, GOT1 and GOT2 catabolized proline to pyruvate, which was completely degraded through TCA cycle (Kukkola et al., 2003). To explore glutamine family amino acid metabolism of eight liver cell types in rat liver regeneration, we isolated eight liver cell types by using the combination of Percoll density gradient centrifugation and immunomagnetic bead methods and the Rat Genome 230 2.0 Array (Xu and Chang, 2008) to detect gene expression changes of eight liver cell types during rat liver regeneration and finally how these genes involved in activities of eight regenerating liver cell types were analysed by methods of bioinformatics and systems biology.

2. Materials and methods

2.1. 2/3 hepatectomy in rat and liver regeneration

A total of 114 cleaning-grade Sprague–Dawley rats, weighing 230 ± 20 g provided by the Animal Center of Henan Normal University, were randomly divided into nine PH groups, nine operation control groups and one NC (normal control) group, six rats in each group (male/female=1:1). Rats in the PH groups underwent operation for removal of 70% of their liver as previously described (Higgins and Anderson, 1931). Briefly, the left and median lateral liver lobes were surgically removed, and the rats after hepatectomy were normally fed for 0, 2, 6, 12, 24, 30, 36, 72, 120 and 168 h, respectively. Rats in the operation control groups received the same treatment as PH groups, but their liver lobes were not removed. The Laws of Animal Protection of China were strictly implemented.

2.2. Isolation and identification of eight liver cell types

Rats were subjected to abdominal skin disinfection with alcohol after anaesthetized by inhaling diethyl ether. The abdominal cavity was opened to expose the liver, and the superior vena cava and the inferior vena cava were ligated followed by portal vein cannulation. Conventional two-step perfusion method was used to separate liver cell suspension. Briefly, the liver was perfused with calcium-free perfusate preheated at 37°C until it turned grey, then with a 15-ml 0.05% collagenase IV solution instead of perfusate at a flow rate of 1 ml/min. After the liver capsule was removed, the perfused liver was cut into small pieces and digested with 0.05% collagenase IV for 15 min at 37°C. After being filtered through the 200-well nylon netting, the liquid was centrifuged at 500 ***g*** for 3 min. Pellet at the bottom was collected and washed three times in a 4°C PBS buffer to adjust the cell concentration to 1×10^8 cells/ml. Six millilitres of the mixed cell suspension was spread on to the surface of 4 ml 60% Percoll in a 10-ml tube for a single centrifugation at 200 ***g*** for 5 min at 4°C. The centrifuged pellets and supernatant were the purified hepatocytes (Vondran et al., 2008) and non-parenchymal cell-enriched supernatant fractions, respectively. The supernatant was mixed with an equal volume of PBS, centrifuged at 400 ***g*** for 2×2 min at 4°C. The mixed non-parenchymal cell-rich pellet collected was adjusted to a concentration of 1×10^8 cells/ml with PBS and mixed with 10 μl/ml of rat anti-THY1, -GFAP, -CK31, -CD68, -CD161a, -CD11c PE-antibodies, respectively. Oval, hepatic stellate, sinusoidal endothelial, Kupffers, pit and dendritic cells were picked out as previously described (Grisham, 1983). On the other hand, white intrahepatic bile duct fractions left on the nylon netting were added into the digestive solution containing 0.25% trypsin and 0.05% collagenase IV, incubated at 37°C for 50 min and filtered through the 200-well nylon netting. The filtered solution was centrifuged at 300 ***g*** for 5 min. The resulting sediment was the pellet enriched with biliary epithelial cells (Blair et al., 1995). The biliary epithelial cells were isolated with rat anti-CK19 PE antibody as previously described. Finally, anti-ALB and G6P, CK18 and GGT1, OC2 and OV6, CD14 and ET-1, LYZ and ED2, DES and VIM, CD8 and CD56, CD86 and CD103 antibodies were used to identify liver, biliary epithelia, oval, hepatic stellate, sinusoidal endothelial, Kupffer, pit and dendritic cells as previously described.

2.3. Rat Genome 230 2.0 Microarray detection and data analysis

Total RNA was isolated from the frozen livers with Trizol reagent (Invitrogen Corporation) following its manufacturer's instructions and purified following the RNeasy Mini protocol (Qiagen, Inc.) (Norton, 1992). The quality of total RNA samples was assessed by

measuring the optical density at 260/280 nm and agarose electrophoresis (Scott, 1995). As a template, 5 μg of total RNA was used to synthesize the first strand of cDNA using SuperScript II RT (Invitrogen Corporation) and T7-oligo dT(24) (W.M. Keck Foundation) as the primer. Second-strand synthesis was performed with the Affymetrix cDNA single-stranded cDNA synthesis kit. The cDNA product was purified following the cDNA purify protocol (Xiao et al., 2008). The 12-μl purified cDNA subsequently served as a template for the production of biotin-labelled cRNA transcript using the GeneChip *in vitro* transcript labelling kit (ENZO Biochemical). The labelled cRNA was purified using the RNeasy Mini Kit columns (Qiagen) (Kube et al., 2007). The concentration, purity and quality of cDNA and cRNA were assessed as above. Fifteen microlitres cRNA (1 μg/μl) was incubated with 6 μl 5× fragmentation buffer and 9 μl RNase-free water for 35 min at 94°C and digested into 35–200 bp cRNA fragments. The prehybridized Rat Genome 230 2.0 Microarray was put into a hybridization buffer prepared following the Affymetrix protocol, and hybridized in a rotating chamber (60 rev./min, 16 h, 45°C). Arrays were washed to remove superfluous hybridization buffer, stained in GeneChip fluidics station 450 (Affymetrix Inc.), scanned with a GeneChip Scanner 3000 (Affymetrix Inc.) to obtain images. The images were converted to signal value using Affymetrix GCOS 1.4 software. The probe signal values were scaled to evaluate gene expression (P-value<0.05), marginal expression ($0.05<P$-value<0.065) and no expression (P-value>0.065). Then, the signal value of each chip was normalized, and the relative values of genes were evaluated with the ratios comparing the normalized P-value in PH groups with that in control groups. For example, the expression of genes with a relative value $\geqslant 3$ is considered up-regulated, the expression of genes with a relative value ≤ 0.33 is considered down-regulated and genes with a relative value of 0.33∼2.99 are considered meaningless genes. To minimize the technical errors from microarray analysis, each sample was analysed at least three times with Rat Genome 230 2.0 Microarray. Their average value was calculated as a corrective value. Finally, these values were analysed using GeneMath, GeneSpring (Silicon Genetics), Microsoft Excel Software (Microsoft) and Pathwaystudio 5.0 (Doniger et al., 2003; Twigger et al., 2006; Wang et al., 2007; Bult et al., 2008; Guo et al., 2008).

2.4. RT-PCR

Primer and probe sequences were designed by Primer Express 2.0 software according to mRNA sequences of eight marker genes *g6pc*, *ggt1*, *oc2*, *gfap*, *cd14*, *lyz*, *cd56*, *cd86* (marker genes of HC, BEC, OC, HSC, SEC, KC, PC and DC, respectively) and housekeeping genes – β-*actin* (GenBank number: U07993, NM_053840, BG671896, NM_017009, NM_021744, L12458, NM_031521, NM_020081 and NM_031144) and synthesized by Shanghai GeneCore BioTechnologies Co., Ltd (Yoon et al., 2002). Then, target genes were subjected to amplifcation and PCR test on PRISM 7900 Sequence Detector (ABI Company) according to the operational guideline manual for QuantiTect SYBR Green RT-PCR Kit (Qiagen), and the copies of target genes in the sample in percent millilitres were calculated according to standard curve, and the relative expression content computed according to β-*actin* copy number (Fleige et al., 2006). The expressions of marker genes *g6pc*, *ggt1*, *oc2*, *gfap*, *cd14*, *lyz*, *cd56* and *cd86* of HC, BEC, OC, HSC, SEC, KC, PC and DC were measured using RT-PCR and were compared with the results of Rat Genome 230 2.0 chip if their gene expression profile were similar, indicating that the results of the chip was reliable (Supplementary Figure S1 at http://www.cellbiolint.org/cbi/034/cbi03411189add.htm).

2.5. Glutamine family amino acid metabolism-associated gene identification

According to the classfication of physiological activity from the website Gene Ontology database (www.geneontology.org), we inputted the terms at NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu) to identify the ralated genes of rat, mouse and human. Influential genes were confirmed based on the biological pathway maps in GENMAPP (www.genmapp.org), KEGG (www.genome.jp/kegg/pathway.html) and BIOCARTA (www.biocarta.com/genes/index.asp) (Osband and Cashon, 1990; Ogata et al., 1999; Doniger et al., 2003; Wang et al., 2007); finally, this reconfirmed 49 genes involved in glutamine family amino acid metabolism. Sequentially, these were compared with genes in the Rat Genome 230 2.0 Array, and it was found that the chip contains 48 of the genes.

2.6. Rat liver regeneration-associated gene identification

After rat partial hepatectomy, the genes increased $\geqslant$3-fold compared with the control in gene expression at least one time point and were identified as significant up-regulated genes; the genes decreased $\leq$3-fold compared with the control in gene expression and were identified as significant down-regulated genes. They were identified as significant up-/down-regulated genes with a significant up-regulation at one time point and down-regulation at another time point. The genes with the same expression trend were verified at least two times at the same time point after three times detection. Significantly expressed genes change, and expression of abundance has significant ($P\leq 0.05$) or very significant ($P\leq 0.01$) differences between the partial hepatectomy group and the control group, and they were referred to as liver regeneration-associated genes.

3. Results

3.1. The expression changes comparison of glutamine family amino acid metabolism-related genes

We investigated gene expression profiles of eight liver cell types during liver regeneration. It showed that genes related to metabolism of glutamate, glutamine, argine and proline were found to have significant expression changes in HC, BEC, OC, HSC, SEC, KC, PC and DC, as can be seen in the following: 7, 1, 6 and 2; 10, 4, 14 and 6; 6, 1, 2 and 3; 7, 1, 4 and 4; 5, 1, 6 and 1; 5,

0, 7 and 1; 6, 1, 8 and 2; 3, 1, 11 and 2, respectively (Figure 1). Among them, *aldh4a1* was specifically expressed in hepatocytes; *arg2*, *glud1*, *gfpt1*, *gmps*, *lap3*, *nos1*, *pycr2* and *rars* were specifically expressed in biliary epithelial cells; *eprs*, *got2* and *nadsyn1* were specifically expressed in hepatic stellate cells; *glu1* was specifically expressed in sinusoidal endothelial cells; the expression of other genes in the eight liver cells have much differences.

3.2. Expression dynamics and interactions of glutamine family amino acid metabolism-related genes

Gene expression changes of glutamine family amino acid metabolism-related genes in eight liver cell types were analysed with Cluster software. It showed that there are two expression patterns including all up-regulated and all down-regulated in HC, KC, PC and DC; three expression patterns including all up-regulation, mostly up-regulation and all down-regulation in OC; three expression patterns including all up-regulation, all down-regulation and mostly down-regulation in HSC and SEC; five expression trends including all up-regulation, mostly up-regulation, all down-regulation, mostly down-regulation, equal frequencies of up- or down-regulation in BEC (Figure 2). Usually, liver regeneration is classified into three stages including priming (PH after 2–6 h), progress (PH after 6–72 h) and termination (PH after 72–168 h) according to the process of liver regeneration and cell physiological activities (Steer, 1995). Interaction relationships of glutamine family amino acid metabolism-related genes were constucted based on the KEGG database: in the priming stage of liver regeneration, glutamate synthesis-promoting genes *prodh* and *gls2* were up-regulated in HSC and SEC, respectively; glutamate degradation-promoting genes *aldh5a1*, *gad2*, *gpt*, *got1* and *got2* were up-regulated in HC, BEC, HSC, SEC, PC and DC; polyamine synthesis-promoting genes *odc1* and *srm* were up-regulated in BEC, SEC and KC; urea cycle promotion genes *arg1*, *ass1* and *asl* were up-regulated in BEC and SEC; glutamyl-tRNA synthesis-promoting gene *ears2* was up-regulated in SEC; NO synthesis-promoting genes *nos2* and *nos3* were up-regulated in SEC and KC; creatine phosphate synthesis-promoting genes *gatm*, *ckm* and *ckmt2* were up-regulated in KC and PC; NO synthesis-promoting genes *nos3* and *nos1* were down-regulated in HC and BEC, respectively; proline degradation-promoting genes *p4ha* and creatine phosphate synthesis-promoting genes *ckm* and *ckmt1* were down-regulated in BEC; proline degradation-promoting gene *prodh2* were down-regulated in PC and DC. In the progress stage of liver regeneration, creatine phosphate synthesis-promoting genes *gatm*, *ckm*, *ckmt1* and *ckb* were up-regulated in HC and BEC; glutamate degradation-promoting genes *gad2*, *aldh5a1* and *pycr1* were up-regulated in BEC and KC; glutamine degradation-promoting genes *gmps*, *gfpt1* and *gfpt2*, urea cycle promotion gene *arg2*, NO synthesis-promoting gene *nos2* and proline degradation-promoting gene *p4ha3* were up-regulated in BEC; proline synthesis-promoting gene *pycr1* and NO synthesis-promoting gene *nos2* were up-regulated in PC; urea cycle promotion genes *arg1* and *asl*, polyamine synthesis-promoting genes *odc1* and *srm* and NO synthesis-promoting gene *nos2* were up-regulated in DC; glutamate synthesis-promoting genes *aldh4a1*, *gls2* and *oat* were down-regulated in HC, BEC and PC; glutamate degradation-promoting genes *got1*, *gpt*, *glud1* and *pycr2* were down-regulated in HC and BEC; NO synthesis-promoting gene *nos3* was down-regulated in HC; urea cycle promotion genes *arg1*, *ass1*, *as*l and *otc* were down-regulated in BEC, PC and DC; creatine phosphate synthesis-promoting genes *ckb* and *ckmt2* were down-regulated in BEC and KC, respectively; proline synthesis-promoting genes *lap3* and *prodh2* were down-regulated in BEC. In the termination stage of liver regeneration, creatine phosphate synthesis-promoting gene *gatm*, glutamate degradation-promoting gene *gad1* and proline degradation-promoting gene *p4ha3* were up-regulated in HC, but other genes which were involved in metabolism were down-regulated in different cells at this stage (Figure 2).

4. Discussion

The study showed that *prodh*, which oxidizes proline to glutamate, expressed up-regulation at 2 h in HSC and at 72 h in OC after PH; *gls2*, which hydrolyses glutamine into glutamate was up-regulated at 2 h in SEC, while the genes which promotes synthesis of glutamate were down-regulated in other cells. The genes which promote degradation of glutamate, *gad1* and *gad2*, were up-regulated at 6 and 72 h in HC; *got1* and *got2* were up-regulated at 2 h in HSC; *got1*, *gpt* and *gad1* were up-regulated at 6–12, 6 and 120 h in SEC, respectively; *got1* almost expressed up-regulation in the entire liver regeneration in DC. *eprs* and *ears2* promoted glutamate to glutamyl-tRNA at 2 h in HSC and at 6 h in SEC, respectively. The results indicated that in the priming stage of liver regeneration, glutamate was synthesised by HSC and SEC from proline and glutamine, respectively; it was catabolized by HC, HSC, SEC and DC; and HSC and SEC transformed it into glutamyl-tRNA for protein synthesis; in the termination stage, glutamate was synthesized in OC and was catabolized in HC and DC.

Glutamine is an important precursor of nucleic acid and energy source. This paper has shown that *glul*, which promotes glutamine synthetase was down-regulated at 36 h in DC, *gmps*, which hydrolyses glutamine to GMP, *gfpt1* and *gfpt2*, which catalysed glutamine to 6-phosphate glucosamine, were up-regulated at 12–24 h in BEC, *gfpt2* was also up-regulated at 36 and 120 h in OC; *nadsyn1* catalysed glutamine to NAD at 36 h in HSC. The results indicated that glutamine was catalysed into GMP in BEC, into glucosamine 6-phosphate in BEC and OC, into NAD in HSC in the progress stage of liver regeneration.

In this paper, the research indicated that *arg1*, which hydrolyses arginine to ornithine, was up-regulated at 6 h in SEC and DC after PH, *arg2* was up-regulated at 12–24 h in BEC; *ass1*, which catalyses synthesis of argininosuccinate with citrulline and succinate, was up-regulated at 2–6 h in BEC; *asl*, which cleaves argininosuccinate to arginine, was up-regulated at 6 h in BEC, at 6–12 h in SEC and at 36 h in DC, while the above-mentioned genes and *otc* were down-regulated at other time points in different cells. *nos2*, which catabolizes arginine to release NO, was up-regulated at 24 h in BEC, at 36 and 120 h in OC, at 2 and

Gene Symbol	Cell Types	Recovery time (h) after partial hepatectomy (PH) 0	2	6	12	24	30	36	72	120	168	Max/M in Folds
1 Glutamate metabolism												
1.1 Glutamate biosynthesis												
	HC	1.00	2.55	3.44	4.49	1.64	2.30	1.51	0.94	0.99	1.14	4.49
Odc1	BEC	1.00	2.72	5.04	1.53	1.06	4.50	2.00	1.36	1.54	1.51	5.04
	KC	1.00	1.58	3.23	3.68	2.28	2.56	1.96	1.40	0.62	0.83	3.68
	DC	1.00	1.54	3.36	5.74	1.72	1.57	1.74	0.92	0.87	0.83	5.74
	HC	1.00	0.81	0.54	0.27	0.28	0.21	0.27	0.14	0.45	0.50	0.21
	BEC	1.00	3.02	1.66	0.05	0.10	0.87	0.40	0.33	0.83	0.30	0.05
Oat	KC	1.00	1.19	0.54	0.38	0.44	0.52	0.70	0.59	0.44	0.32	0.32
	PC	1.00	1.06	0.37	0.54	0.54	0.23	0.58	0.45	0.63	0.86	0.23
	DC	1.00	0.83	1.09	0.87	0.37	0.28	1.03	0.60	0.38	0.77	0.28
Aldh4a1	HC	1.00	0.91	0.60	0.26	0.41	0.40	0.47	0.54	1.00	0.86	0.26
	HC	1.00	1.05	0.66	0.56	0.26	0.30	0.23	0.37	0.69	0.85	0.23
Gls2	BEC	1.00	1.71	2.07	0.06	0.18	0.51	0.60	0.85	1.31	0.83	0.06
	SEC	1.00	1.85	3.90	1.08	0.83	1.32	0.44	1.93	0.56	0.38	3.90
	PC	1.00	1.07	0.42	0.38	0.44	0.41	0.47	0.11	0.38	1.30	0.11
Prodh	OC	1.00	1.42	1.60	1.57	1.76	1.01	0.56	3.60	0.60	0.82	3.60
	HSC	1.00	3.84	0.86	1.32	1.08	1.41	2.67	0.74	0.54	1.10	3.84
	BEC	1.00	0.60	0.82	0.80	1.06	2.67	4.12	1.48	0.96	1.73	4.12
Pycr1	OC	1.00	1.43	1.34	1.73	1.77	5.54	2.63	1.90	3.86	1.50	5.54
	KC	1.00	0.93	0.94	2.13	2.81	3.81	1.77	1.85	0.67	0.49	3.81
	PC	1.00	2.48	1.00	3.28	3.83	2.58	2.23	1.25	0.99	1.25	3.28
Pycr2	BEC	1.00	0.50	0.51	0.29	0.13	1.14	2.00	1.73	0.93	1.57	0.13
1.2 Glutamate catabolism												
Glud1	BEC	1.00	2.15	2.19	0.16	0.12	3.00	1.97	0.62	1.13	0.64	0.12
	BEC	1.00	8.12	16.75	0.32	0.16	2.28	1.00	0.75	1.93	0.70	16.75;0.16
	OC	1.00	4.24	4.75	3.65	1.72	1.04	1.68	2.65	1.05	0.93	4.75
Got1	HSC	1.00	4.63	2.11	2.43	0.83	0.44	0.79	0.42	0.55	0.52	4.63
	SEC	1.00	3.43	26.52	3.53	2.08	1.70	1.18	2.66	0.99	0.77	26.52
	DC	1.00	4.54	3.89	4.82	4.93	1.45	11.84	2.90	4.46	1.16	11.84
Got2	HSC	1.00	3.57	0.88	2.56	1.03	1.21	2.30	1.01	1.06	1.10	3.57
	HC	1.00	0.93	0.90	0.48	0.27	0.46	0.30	0.41	0.52	0.51	0.27
	BEC	1.00	0.62	0.74	0.07	0.05	0.25	0.56	0.64	0.76	0.66	0.05
	OC	1.00	1.08	0.56	0.67	0.69	0.25	0.33	1.95	0.22	0.30	0.22
Gpt	HSC	1.00	2.79	0.88	6.96	0.31	0.49	3.70	0.14	0.28	1.05	6.96;0.14
	SEC	1.00	3.84	8.20	3.95	1.08	2.01	1.58	6.29	1.10	0.51	8.20
	KC	1.00	0.72	1.33	0.92	0.67	0.80	0.45	0.64	0.28	0.26	0.26
	PC	1.00	1.09	0.27	0.60	0.66	0.36	0.37	0.12	0.46	0.68	0.12
Gad1	HC	1.00	1.54	2.20	2.34	2.25	1.22	1.87	3.84	1.46	2.36	3.84
	SEC	1.00	1.18	1.44	1.71	1.72	1.65	1.53	1.89	3.28	2.16	3.28
	HC	1.00	1.03	3.67	7.82	2.66	2.72	4.79	9.55	3.74	2.32	3.67
	BEC	1.00	0.11	0.18	2.09	2.53	0.43	0.75	0.62	0.18	0.82	0.11
Gad2	OC	1.00	0.88	0.74	0.44	0.75	0.12	0.50	1.08	0.35	0.42	0.12
	KC	1.00	1.48	1.44	2.46	0.39	1.77	5.41	1.18	0.96	0.48	5.41
	PC	1.00	1.08	2.32	1.05	1.10	1.80	2.15	1.11	2.49	0.32	0.32
Abat	OC	1.00	0.71	0.49	0.28	0.80	0.75	0.53	0.72	0.59	0.56	0.28
	HSC	1.00	1.05	0.64	0.96	0.88	0.61	1.35	0.34	0.29	0.62	0.29
	BEC	1.00	0.50	0.42	3.09	1.26	0.88	0.99	1.00	0.62	1.03	3.09
Aldh5a1	HSC	1.00	7.09	3.27	4.61	1.34	4.63	9.48	1.19	1.90	3.92	7.09
	PC	1.00	6.20	3.00	1.38	2.84	1.02	2.57	1.73	1.09	1.35	6.20
1.3 L-Glutamyl-tRNA												
Eprs	HSC	1.00	4.62	1.07	4.15	1.24	1.61	2.27	0.81	0.70	1.14	4.62
Ears2	SEC	1.00	1.22	3.00	1.14	1.17	1.63	1.30	2.42	1.42	1.39	3.00
2 Glutamine metabolism												
2.1 Glutamine biosynthesis												
Glul	DC	1.00	0.89	0.66	0.58	0.31	0.46	0.24	0.95	0.84	1.63	0.24
2.2 Glutamine catabolism												
Nadsyn1	HSC	1.00	2.05	1.09	2.35	1.46	1.64	3.62	1.32	1.80	1.72	3.62
Gmps	BEC	1.00	1.06	1.76	3.42	5.02	1.44	1.32	0.98	0.89	0.95	5.02
Gfpt1	BEC	1.00	0.47	0.67	3.26	2.70	0.55	1.04	0.98	0.53	0.80	3.26
Gfpt2	BEC	1.00	0.69	0.78	2.90	3.33	0.87	0.82	0.98	0.76	0.92	3.33
	OC	1.00	2.11	2.66	2.31	1.28	1.44	4.36	1.90	3.29	1.35	4.36
	HC	1.00	1.05	0.66	0.56	0.26	0.30	0.23	0.37	0.69	0.85	0.23
Gls2	BEC	1.00	1.71	2.07	0.06	0.18	0.51	0.60	0.85	1.31	0.83	0.06
	SEC	1.00	1.85	3.90	1.08	0.83	1.32	0.44	1.93	0.56	0.38	3.90
	PC	1.00	1.07	0.42	0.38	0.44	0.41	0.47	0.11	0.38	1.30	0.11
3 Arginine metabolism												
3.1 Urea cycle												
	BEC	1.00	1.59	2.24	0.06	0.03	1.92	1.25	0.74	1.58	0.76	0.03
	HSC	1.00	2.39	1.12	2.47	1.04	0.27	1.47	0.27	0.19	0.53	0.19
Arg1	SEC	1.00	1.87	6.54	2.25	1.02	0.80	0.61	1.53	0.19	0.12	6.54;0.12
	PC	1.00	1.44	0.81	1.54	0.94	0.69	0.81	0.14	0.34	0.85	0.14
	DC	1.00	1.41	3.41	4.39	1.97	1.44	4.75	1.33	1.07	0.39	4.75
Arg2	BEC	1.00	0.57	0.50	6.20	5.69	0.71	1.75	2.05	0.89	0.97	6.20
Otc	HC	1.00	0.94	0.54	0.34	0.16	0.27	0.21	0.20	0.47	0.73	0.20
	BEC	1.00	2.09	2.16	0.01	0.02	0.66	0.66	0.18	0.71	0.23	0.01
	HSC	1.00	0.78	0.98	2.82	0.49	0.26	1.21	0.37	0.17	0.45	0.17
	SEC	1.00	1.72	1.12	0.55	0.16	0.24	0.23	0.67	0.16	0.10	0.10
	KC	1.00	0.98	0.77	0.52	0.61	0.60	0.59	0.77	0.29	0.35	0.29
	PC	1.00	0.84	0.58	0.45	0.35	0.26	0.42	0.06	0.40	0.71	0.06
	DC	1.00	1.05	0.74	1.00	0.31	0.25	1.01	0.50	0.15	0.29	0.15

Figure 1 **The expression change comparison of glutamine family amino acid metabolism-related genes in eight rat regenerating liver cell types**

Figure 1 contd

Gene Symbol	Cell Types	Recovery time (h) after partial hepatectomy (PH) 0	2	6	12	24	30	36	72	120	168	Max/Min Folds
	BEC	1.00	4.44	6.45	0.10	0.10	1.30	0.48	0.51	1.24	0.56	6.45;0.10
	HSC	1.00	0.95	1.87	1.52	0.51	0.21	0.55	0.25	0.38	0.30	0.21
Ass1	SEC	1.00	1.75	4.10	1.47	0.71	0.63	0.35	1.12	0.30	0.19	0.19
	PC	1.00	1.08	0.48	0.56	0.38	0.15	0.47	0.18	0.46	0.80	0.15
	DC	1.00	1.30	2.12	2.02	0.64	0.28	3.21	0.65	1.00	0.43	0.28
	BEC	1.00	2.42	4.30	0.05	0.07	2.78	2.63	0.48	1.65	0.51	4.30;0.05
Asl	HSC	1.00	3.56	1.17	1.88	0.97	0.94	2.31	0.41	0.43	0.61	3.56
	SEC	1.00	2.83	13.77	3.36	2.24	2.63	1.65	2.53	0.80	0.86	13.77
	DC	1.00	1.60	2.02	2.22	2.67	1.69	4.18	1.64	1.15	0.44	4.18
3.2 No												
Nos1	BEC	1.00	0.20	0.18	2.31	2.22	0.44	1.17	0.71	0.33	0.71	0.18
	BEC	1.00	2.32	1.56	2.82	7.15	0.84	2.64	2.47	1.75	2.52	7.15
	OC	1.00	2.73	0.99	1.07	0.23	1.30	3.86	0.74	3.24	0.80	3.86;0.23
Nos2	SEC	1.00	6.24	0.93	6.54	2.63	0.41	0.43	0.74	1.96	6.66	6.66
	KC	1.00	2.82	1.15	1.21	2.33	3.81	1.28	1.69	0.29	2.51	0.29
	PC	1.00	2.72	2.68	4.13	6.86	0.81	3.25	9.42	3.63	0.64	4.13
	DC	1.00	2.36	14.95	2.20	8.27	12.20	2.83	7.14	5.76	1.53	12.20
	HC	1.00	0.24	0.87	0.68	2.29	1.50	1.77	3.71	1.35	1.54	0.24
	KC	1.00	3.37	5.90	2.73	3.49	2.64	4.72	7.64	7.91	3.95	7.91
Nos3	PC	1.00	0.99	1.02	0.56	0.85	0.93	1.34	0.24	0.93	0.51	0.24
	DC	1.00	0.79	1.01	0.20	1.09	0.95	0.29	0.68	0.69	0.49	0.20
3.3 Phosphocreatine												
	HC	1.00	0.51	0.72	0.41	1.76	1.30	1.15	5.89	2.45	2.19	5.89
	BEC	1.00	2.29	2.05	3.30	1.92	0.86	0.95	1.46	2.42	1.52	3.30
Gatm	OC	1.00	1.69	0.80	0.65	1.58	0.53	1.37	1.35	1.28	3.22	3.22
	KC	1.00	2.11	2.91	1.36	1.27	2.84	4.39	5.76	3.61	3.65	5.76
	PC	1.00	1.17	5.19	1.34	1.28	1.04	1.05	1.08	1.06	1.34	5.19
	DC	1.00	1.29	0.61	0.46	0.99	0.81	0.92	3.10	0.69	1.12	3.10
Ckb	HC	1.00	2.80	2.14	0.60	2.05	4.10	3.36	8.24	2.99	5.00	8.24
	BEC	1.00	1.28	0.41	0.44	0.17	0.93	0.49	1.43	1.39	1.63	0.17
Ckm	BEC	1.00	0.30	0.35	1.99	3.06	0.35	0.78	0.61	0.25	0.79	3.06;0.30
	PC	1.00	3.42	6.66	2.53	2.92	1.59	3.75	1.82	3.25	1.92	6.66
	HC	1.00	2.98	3.88	1.16	3.25	1.16	1.94	7.03	1.91	2.02	7.03
Ckmt1	BEC	1.00	0.57	0.12	4.97	11.82	1.38	1.99	0.84	1.33	1.55	11.82;0.12
	DC	1.00	1.33	5.98	2.56	4.98	3.89	1.22	6.32	1.95	0.88	3.89
	KC	1.00	0.56	0.55	1.07	0.84	0.25	0.32	0.89	0.48	0.37	0.25
Ckmt2	PC	1.00	1.05	4.23	1.14	2.13	2.79	1.02	0.59	1.37	1.33	4.23
	DC	1.00	1.81	5.09	0.68	2.44	4.22	2.65	1.55	2.26	0.81	4.22
3.4 Catecholamines												
	HC	1.00	2.55	3.44	4.49	1.64	2.30	1.51	0.94	0.99	1.14	4.49
Odc1	BEC	1.00	2.72	5.04	1.53	1.06	4.50	2.00	1.36	1.54	1.51	5.04
	KC	1.00	1.58	3.23	3.68	2.28	2.56	1.96	1.40	0.62	0.83	3.68
	DC	1.00	1.54	3.36	5.74	1.72	1.57	1.74	0.92	0.87	0.83	5.74
	BEC	1.00	1.69	4.94	1.25	1.05	3.18	1.04	1.47	1.11	1.39	4.94
Srm	SEC	1.00	1.01	3.64	2.85	1.72	1.67	1.41	0.74	0.71	1.18	3.64
	KC	1.00	1.59	3.64	5.07	3.75	3.00	2.10	1.63	0.91	1.13	5.07
	DC	1.00	0.95	3.65	3.62	1.56	1.04	1.32	0.62	0.96	0.92	3.62
3.5 L-Arginyl-tRNA												
Rars	BEC	1.00	1.24	2.89	0.33	0.19	2.09	1.59	1.24	1.34	1.40	0.19
4 Proline metabolism												
4.1 Proline biosynthesis												
Lap3	BEC	1.00	1.37	0.73	0.10	0.12	0.88	0.90	0.82	0.80	0.90	0.12
4.2 Proline catabolism												
P4ha3	HC	1.00	1.14	2.25	2.22	2.18	1.87	1.66	4.87	1.57	1.90	4.87
	BEC	1.00	0.35	0.23	3.76	2.52	0.41	1.39	1.11	0.41	1.11	3.76;0.23
Prodh	OC	1.00	1.42	1.60	1.57	1.76	1.01	0.56	3.60	0.60	0.82	3.60
	HSC	1.00	3.84	0.86	1.32	1.08	1.41	2.67	0.74	0.54	1.10	3.84
	BEC	1.00	0.37	0.35	0.04	0.05	0.31	1.39	0.66	0.75	0.71	0.05
Prodh2	PC	1.00	0.91	0.24	0.87	1.18	1.13	0.57	0.26	0.63	1.35	0.24
	DC	1.00	1.05	0.25	0.70	1.38	0.90	1.08	1.45	1.64	0.60	0.25
	BEC	1.00	0.60	0.82	0.80	1.06	2.67	4.12	1.48	0.96	1.73	4.12
Pycr1	OC	1.00	1.43	1.34	1.73	1.77	5.54	2.63	1.90	3.86	1.50	5.54
	KC	1.00	0.93	0.94	2.13	2.81	3.81	1.77	1.85	0.67	0.49	3.81
	PC	1.00	2.48	1.00	3.28	3.83	2.58	2.23	1.25	0.99	1.25	3.28
Pycr2	BEC	1.00	0.50	0.51	0.29	0.13	1.14	2.00	1.73	0.93	1.57	0.13
Aldh4a1	HC	1.00	0.91	0.60	0.26	0.41	0.40	0.47	0.54	1.00	0.86	0.26
Got1	BEC	1.00	8.12	16.75	0.32	0.16	2.28	1.00	0.75	1.93	0.70	16.75;0.16
	OC	1.00	4.24	4.75	3.65	1.72	1.04	1.68	2.65	1.05	0.93	4.75
	HSC	1.00	4.63	2.11	2.43	0.83	0.44	0.79	0.42	0.55	0.52	4.63
	SEC	1.00	3.43	26.52	3.53	2.08	1.70	1.18	2.66	0.99	0.77	26.52
	DC	1.00	4.54	3.89	4.82	4.93	1.45	11.84	2.90	4.46	1.16	11.84
Got2	HSC	1.00	3.57	0.88	2.56	1.03	1.21	2.30	1.01	1.06	1.10	3.57
4.3 L-Prolyl-tRNA												
Eprs	HSC	1.00	4.62	1.07	4.15	1.24	1.61	2.27	0.81	0.70	1.14	4.62

*Red represents meaningful up-regulated genes; green represents meaningful down-regulated genes.

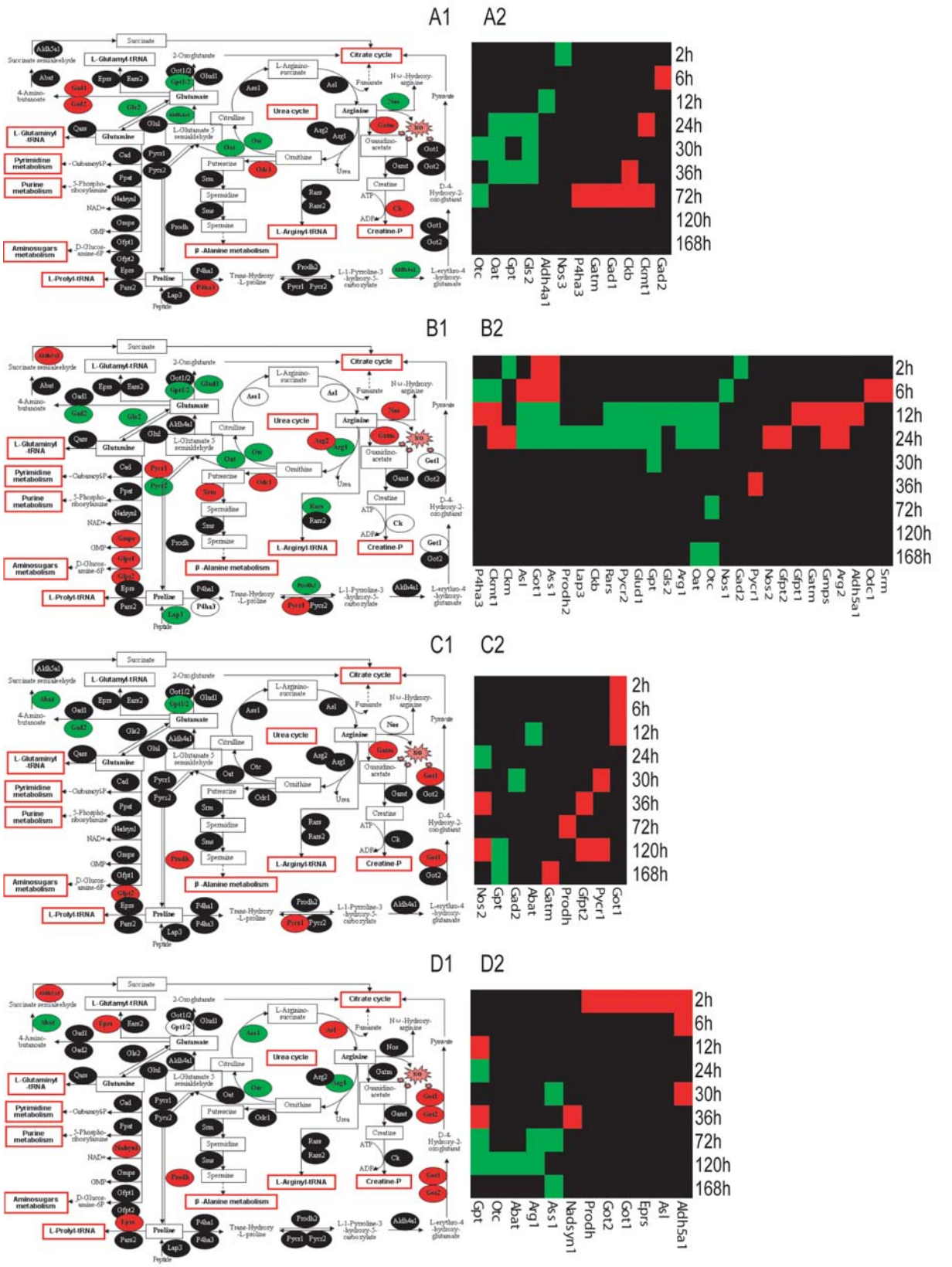
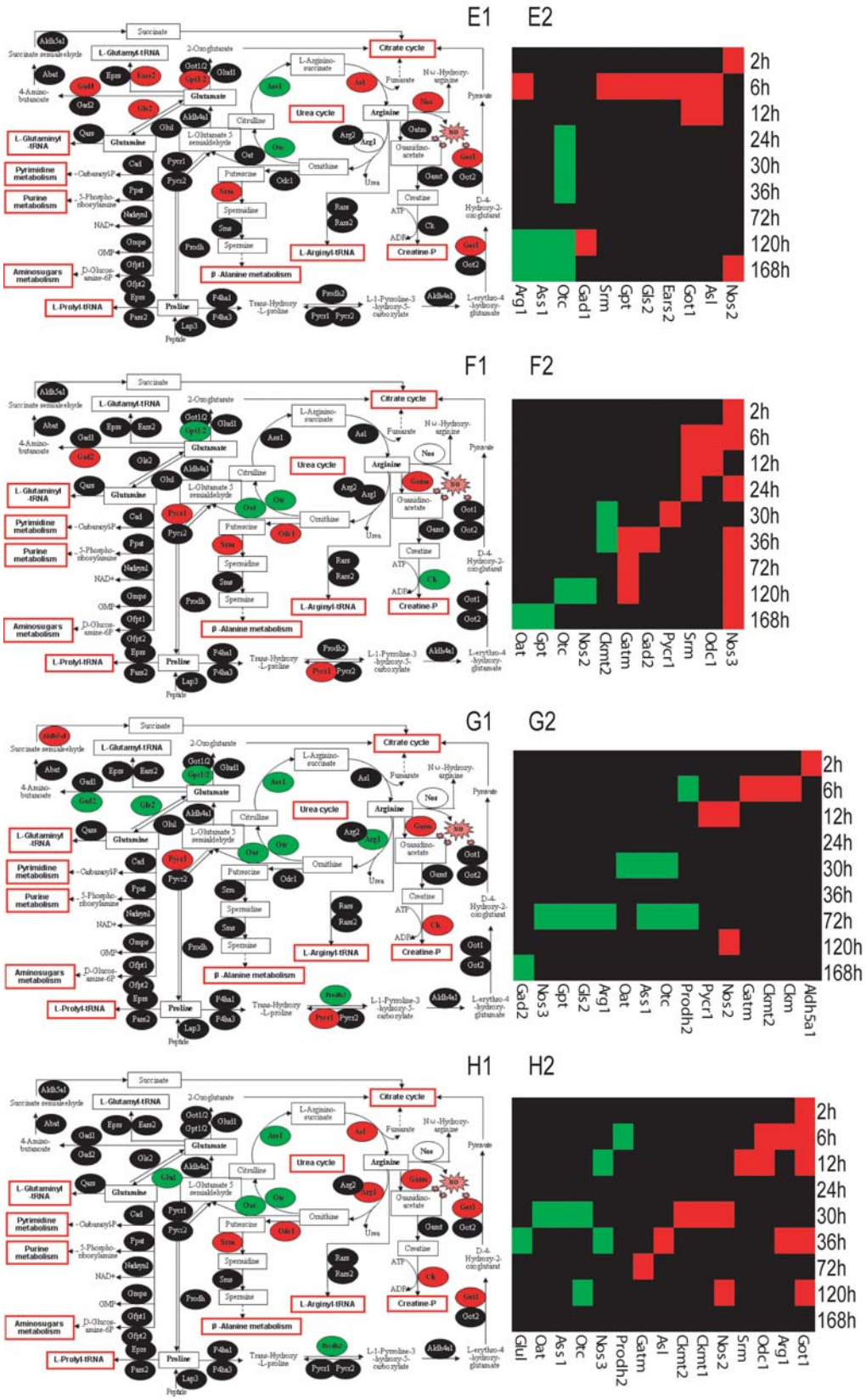


Figure 2 **Expression dynamics and interactions of glutamine family amino acid metabolism-related genes in eight liver cell types in rat liver regeneration**
(**A**) HC; (**B**) BEC; (**C**) OC; (**D**) HSC; (**E**) SEC; (**F**) KC; (**G**) PC; (**H**) DC. Red, green, white and black denote the up-regulated, down-regulated, up-/down-regulated and meaninglessly expressed genes, respectively.

Figure 2 contd



168 h in SEC, at 12 and 120 h in PC, at 30 and 120 h in DC; *nos3* was up-regulated nearly in the entire liver regeneration in DC. *Gatm*, which catalyses arginine to creatine phosphate, was up-regulated at 72 h in HC, at 12 h in BEC, at 168 h in OC, at 36–120 h in KC, at 6 h in PC and at 72 h in DC; *ckb* was up-regulated at 36–72 h in HC; *ckmt1* was up-regulated at 24 and 72 h in HC, at 12–24 h in BEC, at 30 h in DC; *ckmt2* was up-regulated at 6 h in PC and at 30 h in DC. *odc1* and *srm*, which catalyse ornithine to polyamines, were up-regulated at 6–12 h in BEC, SEC, KC and DC. The results show that urea cycle was enhanced in BEC, SEC and DC in the priming stage of liver regeneration; polyamine synthesis was mainly enhanced in BEC, SEC, KC and DC; the release of NO was enhanced in SEC and DC in the priming stage of liver regeneration, in BEC, OC, PC and DC in the progress stage, in OC, SEC, PC and DC in the termination stage; synthesis of creatine phosphate was mainly enhanced in HC, BEC, PC and DC in the progress and termination stages of liver regeneration.

In BEC, *p4ha3*, which oxidizes proline to hydroxyl-L-proline was up-regulated at 72 h in HC and at 12 h, but *prodh2*, which promotes the degradation of proline, was down-regulated in PC and DC. The results above indicated that proline degradation was enhanced in HC and BEC in the priming stage of liver regeneration.

4. Conclusions

In summary, we have shown expression profiles of glutamine family amino acid metabolism at mRNA level in LR by microarray and screened 40 glutamine family amino acid metabolism genes related to liver regeneration in regenerating liver and eight types of liver cells. According to their changes, we found that the LR regulation of complicated glutamine family amino acid metabolism is different in regenerating liver and the eight types of liver cells. However, Rat Genome 230 2.0 chip mainly detects gene transcription, but does not represent expression and activity of protein or enzyme, much less entirely represents glutamine family amino acid metabolism in regenerating liver and the eight types of liver cells. In the future, we will further verify these results by methods such as transgene, Western blot, *in situ* hybridization and so on.

Author contribution

Cuifang Chang is the first author of this paper and is responsible for the experiments, data analysis and article writing. Cunshuan Xu is the corresponding author and is responsible for providing funds, test design and articles review.

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