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ORIGINAL ARTICLE

Expression profiles of the organic acid metabolism-associated genes during rat liver regeneration

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Abstract In this study, 55 of the organic acid metabolism-involved genes were primarily confirmed to be associated with liver regeneration (LR) by bioinformatics and gene expression profiling analysis. Number of the initially and totally expressed genes occurring in initiation phase of LR, G_0/G_1, cell proliferation, cell differentiation and liver tissue structure-function reconstruction were 21, 5, 33, 1 and 40, 20, 174, 44, respectively, illustrating that genes were initially expressed mainly in initiation stage, and worked in different phases. 151 times up-regulation and 114 times down-regulation as well as 14 types of expression patterns showed the diversification and complication of genes expression changes. It is inferred from the above gene expression changes and patterns that acetate biosynthesis enhanced at forepart, propionate biosynthesis at forepart, prophase and early metaphase, pyruvate biosynthesis at forepart, metaphase and anaphase, succinate biosynthesis at forepart and anaphase; malate biosynthesis in metaphase and *N*-acetylneuraminate biosynthesis at 36, 66 and 96 h. Whereas, carnitine biosynthsis attenuates at forepart and prophase, enhancement at middle metaphase; isocitrate in the forepart, quinolinate at forepart and early metaphase, creatine at early metaphase and fumarate at anaphase perform the restrained biosynthesis, respectively; catabolisms of propionate and pyruvate were depressed in metaphase.

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Keywords Partial hepatectomy · Rat genome 230 2.0 array · Organic acid metabolism · Genes associated with liver regeneration

Abbreviations
LR Liver regeneration
PH Partial hepatectomy
SO Sham operation

Introduction

Liver is one of the most important organs in material metabolism (Michalopoulos and DeFrances 2005). After partial hepatectomy (PH) (Higgins and Anderson 1931), the remnant hepatocytes are induced from quiescent state into rapid growth state for compensatory proliferation, which is called liver regeneration (LR) (Fausto et al. 2006; Xu et al. 2004). LR involves many processes such as cellular activation, cell proliferation and its regulation, structure-function reorganization and cell metabolism, etc. (Pahlavan et al. 2006). Presumably that organic acid metabolism is also necessary in the above process. Studies show that metabolism including organic acids metabolism comprises a set of sophisticated metabolic pathways, which are series of consecutive enzymatic reactions that produce specific products (Chou et al. 2006). Enzymes, namely proteins, play a key role in the above enzymatic reactions, and proteins, as the embodiment of life, involved many biochemical events, such as different inherent rule of codon usage in proteins may represent different biological implications (Zhang and Chou 1993, 1994, 1996); versatile architectures have made proteins possess varieties of functions (Chou 2005).

Previous studies have showed that citrate synthase, isocitrate dehydrogenase 1, succinate-CoA ligase (GDP-forming) alpha subunit, fumarate hydratase and malate dehydrogenase promote biosynthesis of succinate, malate, citrate, isocitrate, fumarate and pyruvate in the citric acid cycle, respectively. Catalysis of guanidinoacetate methyltransferase results in synthesis of creatine. Quinolinate was synthesized under the catalytic effects of kynurenine 3-m onooxygenase, kynureninase (Alberati-Giani et al. 1996) and 3-hydroxyanthranilate 3, 4-dioxygenase. Butyrobetaine (gamma), 2-oxoglutarate dioxygenase 1 promotes carnitine biosynthesis. *N*-acetylneuraminate was catalyzed by CMP-*N*-acetylneuraminic acid synthetase. Synthesis of propionate was catalyzed by acetyl-CoA carboxylase alpha and acetyl-CoA dehydrogenase, medium chain, etc. Catalytic synthesis of Pyruvate was supported by pyruvate kinase (liver and RBC), lactate dehydrogenase A-like 6B (Tsuji et al. 1994), etc. Many diseases, such as Glutaric aciduria, Glutaryl-CoA dehydrogenase deficiency, are attributable to the disturbance of organic acid metabolism (Kolker et al. 2004; Varadkar and Surtees 2004). To investigate these biochemical events in rat LR at transcriptional level (Xu et al. 2005; Dransfeld et al. 2005), we employed the Rat Genome 230 2.0 array (Collins 2006) embracing the 106 genes related to organic acid metabolism to check genes expression profiles in regenerating liver after 2/3 hepatectomy based on querying organic acid metabolism-associated genes using the bioinformatics tools (Chou 2004; Cai and Chou 2005; Xu and Chang 2007; Shen and Chou 2007), and found that 55 genes is LR-associated. Their expression changes and correlation with LR as well as function were primarily analyzed (Yue et al. 2001).

Materials and methods

Regenerating liver preparation

Healthy SD rats (200–250 g) were obtained from the Animal Center of Henan Normal University. The 264 rats were separated into groups randomly and one group included six rats. PH was performed according to Higgins and Anderson (1931), the left and middle lobes of liver were removed. Rats were killed by cervical vertebra dislocation at 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 after PH and the regenerating livers were observed at corresponding time point. The livers were wished three times in PBS at 4°C, then 100–200 mg livers from middle parts of right lobe (total mass: 0.1–0.2 g × 6 = 0.6–1.2 g) were gathered and stored at −80°C until use. The sham-operation (SO) groups were the same with PH ones but without liver removal. The laws of animal protection of China were enforced strictly.

RNA isolation and purification

Total RNA was isolated from frozen livers according to the manual of Trizol reagent (Invitrogen Corporation, Carlsbad, CA, USA) (Knepp et al. 2003) and then purified base on the guide of RNeasy mini kit (Qiagen Inc., Valencia, CA, USA) (Nuyts et al. 2001). Total RNA samples were checked to exhibit a 2:1 ratio of 28 S rRNA to 18 S rRNA intensities by agarose electrophoresis (180 V, 0.5 h). Total RNA concentration and purity were estimated by optical density measurements at 260/280 nm (Arkin et al. 1998).

cDNA, cRNA synthesis and purification

cDNA, cRNA purification were based on the way established by Affymetrix. 1–8 μg total RNA as template was used for cDNA synthesis. cRNA labeled with biotin was synthesized utilizing 12 μl cDNA as the template, and cDNA and cRNA were purified according to the purification procedure of GeneChip Analysis (Li et al. 2005). Measurement of cDNA, cRNA concentration and purity were the same as above (Arkin et al. 1998).

cRNA fragmentation and microarray detection

15 μl (1 μg/μl) cRNA incubated with 6 μl 5 × fragmentation buffer at 94°C for 35 min was digested into 35–200 bp fragments. The hybridization buffer prepared according to the way Affymetrix provided was added to the prehybridized Rat Genome 230 2.0 microarray produced by Affymetrix, then hybridization was carried out at 45°C for 16 h on a rotary mixer at 60 rpm. The microarray was washed and stained by GeneChip fluidics station 450 (Affymetrix Inc., Santa Clara, CA, USA). The chips were scanned by GeneChip Scan 3000 (Affymetrix Inc.), and the signal values of gene expression were observed (Collins 2006).

Microarray data analysis

The normalized signal values, signal detections (P, A, M) and experiment/control (Ri) were obtained by quantifying and normalizing the signal values using GCOS (GeneChip operating software) 1.2 (Collins 2006).

Normalization of the microarray data

To minimize the technical error from the microarray analysis, each sample was hybridized three times to the gene chips. The average value of three measurements was normalized, and statistics and cluster analyses were conducted on these values with GeneMath, GeneSpring (Silicon Genetics, San Carlos, CA, USA) and Microsoft Excel Software (Microsoft, Redmond, WA, USA) (Collins 2006; Eisen et al. 1998; Werner 2001).

Identification of genes associated with liver regeneration

The nomenclatures of organic acid biosynthesis or catabolism were adopted from the GENEONTOLOGY database (http://www.geneontology.org), and inputted into at NCBI (http://www.ncbi.nlm.nih.gov) and RGD (http://www.rgd.mcw.edu) to identify the rat, mouse and human genes associated with organic acid metabolism. In addition, according to maps of biological pathways embodied by GENMAPP (http://www.genmapp.org), KEGG (http://www.genome.jp/kegg/pathway.html) and BIOCARTA (http://www.biocarta.com/genes/index.asp), the genes associated with organic acid metabolism were collected, and reconfirmed through literature searches of the pertinent articles. Besides the rat genes, the genes which were now thought to only exist in mouse and/or human and which exhibited a greater than twofold change in the rat regenerating livers were referred to as rat homologous genes. The genes that displayed reproducible results with the three independent analyses and that showed a greater than twofold change in expression in at least one time point as a significant difference ($P \leq 0.05$) or an extremely significant difference ($P \leq 0.01$) between PH and SO, were considered being associated with LR.

Results

Expression changes of the genes associated with organic acid metabolism during liver regeneration

According to the data of databases at NCBI, GENMAPP, KEGG, BIOCARTA and RGD, organic acid metabolism involves 124 genes, and among them, 106 genes were contained in the Rat Genome 230 2.0 array. Fifty-five of them displayed meaningful changes in expression at least in one time point post-PHx, showed significant or extremely significant differences in expression when comparing PH with SO, and exhibited the reproducible result with three independent analysis with Rat Genome 230 2.0 array, suggesting that the genes were associated with LR. It was indicated that there were 23 up-regulated genes, 16 down-regulated genes and 16 up/down-regulated genes during LR. The range of up-regulation was from 2-fold to 30-fold compared with control, and that of down-regulation was 2-fold to 222-fold (Table 1).

Initial expression and total expression profiles of the genes associated with organic acid metabolism during liver regeneration

At each time point of LR, the numbers of initially up-, down-regulated and totally up-, down-regulated genes were in sequence: both 7 and 3 at 0.5 h; 3, 2 and 9, 4 at 1 h; 2, 2 and 6, 5 at 2 h; 0, 2 and 6, 4 at 4 h; 0, 3 and 3, 7 at 6 h; 1, 4 and 4, 12 at 8 h; 0, 4 and 3, 13 at 12 h; 4, 6 and 5, 12 at 16 h; 2, 0 and 7, 7 at 18 h; 0, 0 and 9, 5 at 24 h; 1, 1 and 8, 5 at 30 h; 1, 0 and 7, 9 at 36 h; 2, 1 and 12, 5 at 42 h; 0, 1 and 8, 9 at 48 h; 0, 0 and 6, 1 at 54 h; 0, 0 and 6, 0 at 60 h; 2, 0 and 11, 0 at 66 h; 0, 0 and 6, 0 at 72 h; 1, 0 and 7, 4 at 96 h; 0, 0 and 6, 3 at 120 h; 0, 0 and 8, 2 at 144 h; 0, 0 and 7, 1 at 168 h (Fig. 1). There were totally 26 initially up-regulated and 29 initially down-regulated genes during LR, i.e. at the initiation phase of LR (0.5–4 h after PH), G_0/G_1 transition (4–6 h after PH), cell proliferation (6–66 h after PH), cell differentiation and structure-function reconstruction (72–168 h after PH), the number of initially up and initially down-regulated genes were 12 and 9, 0 and 5, 13 and 20, 1 and 0, respectively. The total number of the up- and down-regulated genes in LR was 151 and 114, and expression frequencies of total 55 genes in the above four phases of LR were 28 and 16, 9 and 11, 89 and 85, 34 and 10, respectively (Fig. 1).

Expression patterns of the genes associated with organic acid metabolism during liver regeneration

According to the function and expression profiles of gene during LR, classification of the expression patterns of total 55 genes into 14 types (Fig. 2) are as follows. (1) acetate biosynthesis, involving one up-regulated gene two down-, and three up/down-; (2) propionate biosynthesis, three up- and two down-; (3) propionate catabolism, three up-regulated, three down-, and one up/down-; (4) pyruvate biosynthesis, seven up-regulated and two up/down-; (5) pyruvate catabolism, one up-regulated and four down-; (6) succinate biosynthesis, two up-regulated; (7) malate biosynthesis, three up-regulated; (8) citrate biosynthesis, two up-regulated and two down-; (9) isocitrate biosynthesis, one up-regulated, one down- and one up/down-; (10) fumarate biosynthesis, one down- and two up/down-; (11) creatine biosynthesis, one down-regulated and one up/down-; (12)

Table 1 Expression abundance of 55 organic acid metabolism-associated genes during rat liver regeneration

Name	Gene abbreviations	Associated to others	Fold difference	Name	Gene abbreviations	Associated to others	Fold difference
1 Acetate biosynthesis				Succinate-CoA ligase, GDP-forming, alpha subunit	Suclg1		0.2
Aspartoacylase	Aspa		4.3	8 Malate biosynthesis			
Acetyl-CoA synthetase 2 (AMP forming)-like	Acas2l		0.3	Malate dehydrogenase, mitochondrial	Mor1		8.9
Aldehyde dehydrogenase family 1, subfamily A4	Aldh 1a4	8	0.1	Malic enzyme 2, NAD(+)-dependent, mitochondrial	Me2	4	5.7
Acylphosphatase 1, erythrocyte (common) type	Acyp1		4.0, 0.1	Malic enzyme 1	Me1	4	2.5
Aldehyde dehydrogenase 1 family, member B1	Aldh1b1	8	2.6, 0.2	9 Citrate biosynthesis			
Cytosolic acetyl-CoA hydrolase	Cach		2.0, 0.1	Citrate synthase	Cs		2.1
2 Propionate biosynthesis				Aconitase 1	Aco1	11	0.5
Acetyl-CoA dehydrogenase, medium chain	Acadm		6.9	10 Related with citrate metabolism			
Acetyl-CoA carboxylase alpha	Acaca	5	3.5	Hydroxyacid oxidase 2 (long chain)	Hao2		26.3
4-Aminobutyrate aminotransferase	Abat	3, 18	2.1	Polymerase (RNA) III polypeptide H	Polr3h		2.9, 0.4
Hydroxyacyl-CoA dehydrogenase/ 3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit	Hadha	3	0.5	11 Isocitrate biosynthesis			
3 Propionate catabolism				Aconitase 1	Aco1	9	0.5
4-Aminobutyrate aminotransferase	Abat	2, 18	2.1	Isocitrate dehydrogenase 1 (NADP+), soluble	Idh1		2.4, 0.2
Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thioalse/enoyl-CoA hydratase (trifunctional protein), alpha subunit	Hadha	2	0.5	12 Fumarate biosynthesis			
24-Dehydrocholesterol reductase	Dhcr24		0.1	Fumarate hydratase	Fh		5.3, 0.2
Solute carrier family 27 (fatty acid transporter), member 2	Slc27a2		2.1, 0.5	13 Creatine biosynthesis			
4 Pyruvate biosynthesis				Guanidinoacetate methyltransferase	Gamt		0.5
Lactate dehydrogenase A-like 6B	Ldhal6b		29.9	Creatine kinase, brain	Ckb		2.4, 0.2
Hydroxyacylglutathione hydrolase-like	Haghl		10.7	14 Carnitine biosynthesis			
Pyruvate kinase, liver and RBC	Pklr		6.6	SET domain, bifurcated 1	Setdb1		3.0
Malic enzyme 2, NAD(+)-dependent, mitochondrial	Me2	8	5.7	Aldehyde dehydrogenase family 1, subfamily A4	Aldh1a4	1	0.1
Lactate dehydrogenase 3, C chain	Ldhc		4.2	Nuclear receptor binding SET domain protein 1	Nsd1		0.1
Malic enzyme 1	Me1	8	2.5	Butyrobetaine (gamma), 2-oxoglutarate dioxgenase 1	Bbox1		6.0, 0.4
Lactate dehydrogenase D	Ldhd		2.1	OUT domain, ubiquitin aldehyde binding 2	Otub2		3.9, 0.3

Table 1 continued

Name	Gene abbreviations	Associated to others	Fold difference	Name	Gene abbreviations	Associated to others	Fold difference
Lactate dehydrogenase B	Ldhb		2.1, 0.4	Suppressor of variegation 3-9 homolog 2	Suv39h2		3.5, 0.4
Hydroxyacyl glutathione hydrolase	Hagh		2.0, 0.3	Myeloid/lymphoid or mixed-lineage leukemia 3	MII3		3.2, 0.4
5 Pyruvate catabolism				Aldehyde dehydrogenase 1 family, member B1	Aldh1b1	1	2.6, 0.2
Acetyl-CoA carboxylase alpha	Acaca	2	3.5	15 *N*-acetylneuraminate biosynthesis			
Pyruvate dehydrogenase complex, component X	Pdhx		0.4	5′,3′-nucleotidase, mitochondrial	Nt5m		3.2
Glyoxylate reductase/ hydroxypyruvate reductase	Grhpr		0.4	Myotubularin related protein 6	Mtmr6		2.6
Pyruvate carboxylase	Pc		0.3	Cytidine monophospho-*N*-acetylneuraminic acid synthetase	Cmas	16	2.3
Pyruvate dehydrogenase (lipoamide) beta	Pdhb		0.2	Phosphohistidine phosphatase 1	Phpt1		2.3
6 Related with pyruvate metabolism				Myotubularin related protein 7	Mtmr7		0.5
Phosphoenolpyruvate carboxykinase 1	Pck1		3.2	16 *N*-acetylneuraminate catabolism			
Aldo-keto reductase family 1, member B7	Akr1b7		0.0045	Cytidine monophospho-*N*-acetylneuraminic acid synthetase	Cmas	15	2.3
Aldo-keto reductase family 1, member B8	Akr1b8		6.5, 0.4	17 Quinolinate biosynthesis			
7 Succinate biosynthesis				Kynurenine 3-monooxygenase	Kmo		0.2
Aldehyde dehydrogenase family 5, subfamily A1	Aldh5a1	18	5.4	Kynureninase	Kynu		0.1
Succinate-CoA ligase, ADP-forming, beta subunit	Sucla2		2.6	3-Hydroxyanthranilate 3,4-dioxygenase	Haao		3.6, 0.4
				18 Butyrate metabolism			
				Butyryl CoA synthetase 1	Bucs1		7.8
				Aldehyde dehydrogenase family 5, subfamily A1	Aldh5a1	7	5.4
				4-Aminobutyrate aminotransferase	Abat	2, 3	2.1

carnitine biosynthesis, one up-regulated, two down- and five up/down-; (13) *N*-acetylneuraminate biosynthesis, four up-regulated and one down-; (14) quinolinate biosynthesis, two down-regulated and one up/down-regulated (Fig. 2).

Discussion

Organic acids, the intermediate metabolites of many substances, have significant biological actions. Among acetate biosynthesis-promoting genes, *aspa* was up-regulated at 0.5–4, 48 h (Namboodiri et al. 2000); *aldh1a4* at 0.5–4, 8, 24 and 48 h, *aldh1b1* at 4, 8 and 18–24 h, *cach* at 8–18 and 36 h and *acas2l* at 48 h were down-regulated, while *acyp1* was up/down-regulated. As for the genes contributing to propionate biosynthesis, the expression levels of three genes *acaca* (Zhang and Kim 1996), *abat* and *acadm* mRNA were increased, however, *hadha* showed downtrend at 0.5, 36 h. *dhcr24* and *slc27a2*, the genes accelerating

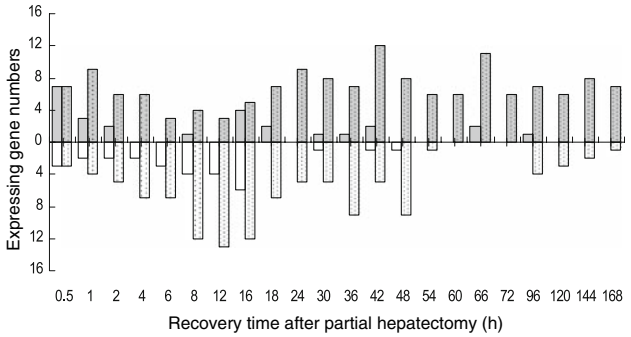

Fig. 1 The initial and total expression profiles of 55 genes associated with organic acid metabolism at each time point of liver regeneration. *X*-axis: Recovery time after partial hepatectomy, *Y*-axis: The number of the genes with the measurable expression changes of every time point. *Grey bars*: Up-regulated gene; *White bars*: Down-regulated. *Blank bars*: Initial expressing genes, *Dotted bars*: The total expressing genes. Gene expression changes span the whole liver regeneration. Initial up-regulated genes dominate at 0.5–2, 18, 30–42, 66 and 96 h, initial down-regulated genes predominate at 4–16 and 48 h, no genes display initial expression at other time points

propionate degradation, displayed down-regulation and up/down-regulation in LR. It is inferred from expression changes of the above genes that there exhibit an increase in acetate biosynthesis in the forepart, propionate biosynthesis in the forepart, prophase and early metaphase, degradation of propionate were blocked middle metaphase.

Among the genes positively regulating pyruvate biosynthesis, expect for down-regulation of genes *hagh* at 8–12 h and *idhb* at 16 h, ten genes including *pklr* were up-regulated at the interval of 0.5–6 and 18–168 h (Wang et al. 2002). In addition, Multi-time down-regulation of five pyruvate catabolism-prompting genes such as *pc* during 8–48 h (Jitrapakdee et al. 1996); of three genes enhancing succinate biosynthesis, *suclg1* being down-regulated at 16, 30, 42 and 96 h, *sucla2* at 18 h and *aldh5a1* at 0.5–6, 54–72 and 120–168 h (Mehta et al. 2006) being up-regulated; genes *me1*, *me2* and *mor1*, whose expression magnified at 42 h, at 18–36 h and at 30, 42 and 96 h (Chow et al. 2005), respectively, belong to the malate biosynthesis-accelerating genes; two citrate biosynthesis-promoting genes *cs* and *aco1* were in a sequence expressed significantly increased at 16 h and decreased at 12 h; isocitrate biosynthesis-enhancing genes *aco1* 12 h and *idh1* at 4–12 h both exhibited the low levels in expression (down-regulated more than twofold); down-regulation of the fumarate biosynthesis-promoting gene *fh* at 16, 36, 48 and 120–144 h (Coustou et al. 2006), which speculates that pyruvate biosynthesis in the forepart, metaphase and anaphase; degradation of pyruvate was blocked at metaphase; succinate biosynthesis in the forepart and anaphase; malate biosynthesis in the metaphase. Whereas, isocitrate biosynthsis shows an decrease in the forepart and fumarate at anaphase. Noticeably, the increased expression of genes *ldhal6b* and *mor1* spanned almost during the entire LR, and reached peaks with 29.9- and 8.9-fold of the control at 18 h and at 42 h, respectively, which indicate that the two genes play key roles separately in pyruvate biosynthesis and malate biosynthesis in corresponding time of LR.

Both *gamt* and *ckb*, the creatine biosynthesis-promoting genes, were down-regulated at 12–18 h (Braissant et al. 2005; Shen et al. 2002), eight genes including *bbox1*, the carnitine biosynthesis-enhancing genes, had multiple-time down-regulations within the period of 0.5–12 h, while trended up at 24–48 h and took on a tendency to change into zigzag form; genes *cmas*, *nt5m*, *phpt1*, *mtmr6* and *mtmr7* have a promotive effect on *N*-acetylneuraminate biosynthesis, expect for downtrend of *cmas* (Tanner et al. 2005) in expression change, the latter four genes showed down-regulation; three genes such as *kmo*, etc., playing the role in quinolinate biosynthesis, were decreased in expression level at 6–24 h (Ito et al. 2004), presuming creatine biosynthesis attenuated at early metaphase; carnitine biosynthsis shows an decrease in the forepart and prophase and enhancement at middle metaphase; *N*-acetylneuraminate biosynthesis swelled at 36, 66 and 96 h; quinolinate biosynthesis weaken at forepart and early metaphase. It is well known that carnitine is helpful for transportation of energy substance required for cell proliferation (Lai et al. 1998), its biosynthesis showed enhancement at middle metaphase, which might support energy for cell proliferation.

In summary, the treatment of experimental material in this study is marked with long times and multiple time points, and expression changes of genes related to organic acid metabolism after rat PH were detected with high-throughput gene expression analysis at transcriptional level. It was primarily proved that there exhibit an increase in acetate biosynthesis in the forepart, propionate biosynthesis in the forepart, prophase and early metaphase, pyruvate biosynthesis in the forepart, metaphase and anaphase, succinate biosynthesis in the forepart and anaphase; malate biosynthesis in the metaphase and *N*-acetylneuraminate biosynthesis at 36, 66 and 96 h. Whereas, carnitine biosynthsis shows an decrease in the forepart and prophase and enhancement at middle metaphase; at the same time, isocitrate in the forepart, quinolinate at forepart and early metaphase, and creatine at early metaphase perform the restrained biosynthesis, respectively; in addition, degradation of pyruvate and propionate were blocked at metaphase. However, DNA → mRNA → protein → function are influenced by many factors including protein

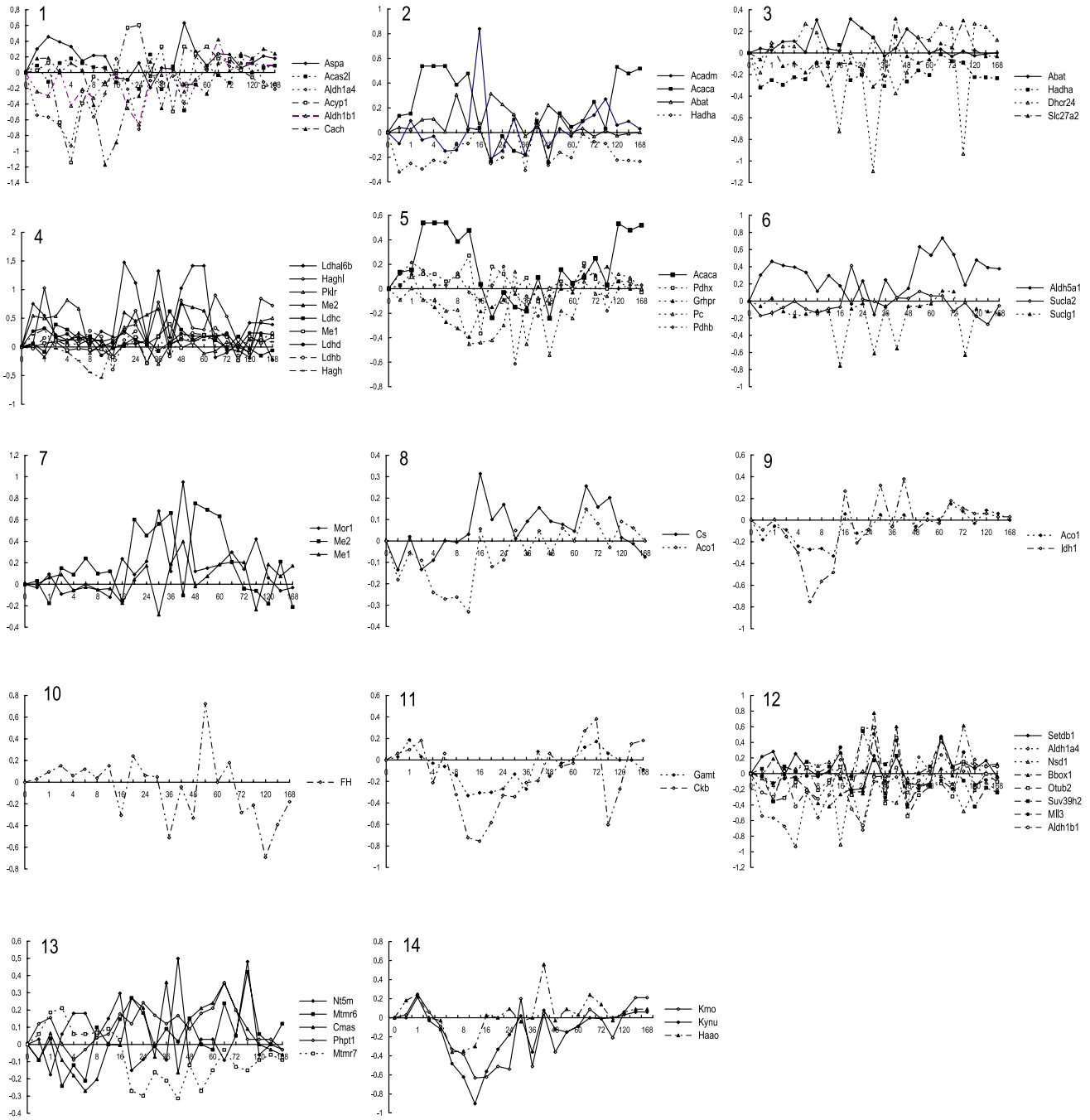

Fig. 2 Expression patterns of 55 genes associated with organic acid metabolism during LR Expression patterns were obtained by the analysis of detection data of Rat Genome 230 2.0 array with Microsoft Excel. *1* acetate biosynthesis; *2*,*3* propionate metabolism; *4*,*5* pyruvate metabolism; *6* succinate biosynthesis; *7* malate biosynthesis; *8* citrate biosynthesis; *9* isocitrate biosynthesis; *10* fumarate biosythesis; *11* creatine biosynthesis; *12* carnitine biosynthesis; *13* *N*-acetylneuraminate biosynthesis; *14* Quinolinate biosynthesis. *Real lines*: the up-regulated genes; *broken lines*: the down-regulated genes; *real-broken lines*: the up/down-regulated genes. *X*-axis indicates the recovery time (h) after PH; *Y*-axis shows logarithm ratio of the signal values of genes at each time point to control

interaction. Therefore, the above results need to be further analyzed with the techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction, etc.

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References

Alberati-Giani D, Buchli R, Malherbe P, Broger C, Lang G, Kohler C, Lahm HW, Cesura AM (1996) Isolation and expression of a cDNA clone encoding human kynureninase. Eur J Biochem 239:460–468

Arkin A, Ross J, McAdams HH (1998) Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected Escherichia coli cells. Genetics 149:1633–1648

Braissant O, Henry H, Villard AM, Speer O, Wallimann T, Bachmann C (2005) Creatine synthesis and transport during rat embryogenesis: spatiotemporal expression of AGAT, GAMT and CT1. BMC Dev Biol 5:9

Cai YD, Chou KC (2005) Using functional domain composition to predict enzyme family classes. J Proteome Res 4:101–111

Chou KC (2004) Molecular therapeutic target for type-2 diabetes. J Proteome Res 3:1284–1288

Chou KC (2005) Using amphiphilic pseudo amino acid composition to predict enzyme subfamily classes. Bioinformatics 21:10–19

Chou KC, Cai YD, Zhong WZ (2006) Predicting networking couples for metabolic pathways of Arabidopsis. EXCLI J 5:55–65

Chow KM, Ma Z, Cai J, Pierce WM, Hersh LB (2005) Nardilysin facilitates complex formation between mitochondrial malate dehydrogenase and citrate synthase. Biochim Biophys Acta 1723:292–301

Coustou V, Biran M, Besteiro S, Riviere L, Baltz T, Franconi JM, Bringaud F (2006) Fumarate is an essential intermediary metabolite produced by the procyclic trypanosoma brucei. J Biol Chem 281:26832–26846

Collins JF (2006) Gene chip analyses reveal differential genetic responses to iron deficiency in rat duodenum and jejunum. Biol Res 39:25–37

Dransfeld O, Gehrmann T, Kohrer K, Kircheis G, Holneicher C, Haussinger D, Wettstein M (2005) Oligonucleotide microarray analysis of differential transporter regulation in the regenerating rat liver. Liver Int 25:1243–1258

Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci 95:14863–14868

Fausto N, Campbell JS, Riehle KJ (2006) Mechanisms of liver regeneration and their clinical implications. Hepatol 43:45–53

Higgins GM, Anderson RM (1931) Experimental pathology of the liver: restoration of the liver of the white rat following partial surgical removal. J Arch Pathol 12:186–222

Ito M, Ogawa K, Takeuchi K, Nakada A, Heishi M, Suto H, Mitsuishi K, Sugita Y, Ogawa H, Ra C (2004) Gene expression of enzymes for tryptophan degradation pathway is upregulated in the skin lesions of patients with atopic dermatitis or psoriasis. J Dermatol Sci 36:157–164

Jitrapakdee S, Booker GW, Cassady AI, Wallace JC (1996) Cloning, sequencing and expression of rat liver pyruvate carboxylase. Biochem J 316:631–637

Knepp JH, Geahr MA, Forman MS, Valsamakis A (2003) Comparison of automated and manual nucleic acid extraction methods for detection of enterovirus RNA. J Clin Microbiol 41:3532–3536

Kolker S, Koeller DM, Sauer S, Horster F, Schwab MA, Hoffmann GF, Ullrich K, Okun JG (2004) Excitotoxicity and bioenergetics in glutaryl-CoA dehydrogenase deficiency. J Inherit Metab Dis 27:805–812

Lai HS, Chen Y, Chen WJ (1998) Carnitine contents in remnant liver, kidney, and skeletal muscle after partial hepatectomy in rats: randomized trial. World J Surg 22:42–47

Li L, Roden J, Shapiro BE, Wold BJ, Bhatia S, Forman SJ, Bhatia R (2005) Reproducibility, fidelity, and discriminant validity of mRNA amplification for microarray analysis from primary hematopoietic cells. J Mol Diagn 7:48–56

Mehta AK, Gould GG, Gupta M, Carter LP, Gibson KM, Ticku MK (2006) Succinate semialdehyde dehydrogenase deficiency does not down-regulate gamma-hydroxybutyric acid binding sites in the mouse brain. Mol Genet Metab 88:86–89

Michalopoulos GK, DeFrances M (2005) Liver regeneration. Adv Biochem Eng Biotechnol 93:101–134

Namboodiri MA, Corigliano-Murphy A, Jiang G, Rollag M, Provencio I (2000) Murine aspartoacylase: cloning, expression and comparison with the human enzyme. Brain Res Mol Brain Res 77:285–289

Nuyts S, Van Mellaert L, Lambin P, Anne J (2001) Efficient isolation of total RNA from clostridium without DNA contamination. J Microbiol Methods 44:235–238

Pahlavan PS, Feldmann RE Jr, Zavos C, Kountouras J (2006) Prometheus' challenge: molecular, cellular and systemic aspects of liver regeneration. J Surg Res 134:238–251

Shen HB, Chou KC (2007) EzyPred: a top-down approach for predicting enzyme functional classes and subclasses. Biochem Biophys Res Comm 364:53–59

Shen W, Willis D, Zhang Y, Schlattner U, Wallimann T, Molloy GR (2002) Expression of creatine kinase isoenzyme genes during postnatal development of rat brain cerebellum: evidence for transcriptional regulation. Biochem J 367:369–380

Tanner ME (2005) The enzymes of sialic acid biosynthesis. Bioorg Chem 33:216–228

Tsuji S, Qureshi MA, Hou EW, Fitch WM, Li SS (1994) Evolutionary relationships of lactate dehydrogenases (LDHs) from mammals, birds, an amphibian, fish, barley, and bacteria. Proc Natl Acad Sci USA 91:9392–9396

Varadkar S, Surtees R (2004) Glutaric aciduria type I and kynurenine pathway metabolites: a modified hypothesis. J Inherit Metab Dis 27:835–842

Wang H, Chu W, Das SK, Ren Q, Hasstedt SJ, Elbein SC (2002) Liver pyruvate kinase polymorphisms are associated with type 2 diabetes in northern European Caucasians. Diabetes 51:2861–2865

Werner T (2001) Cluster analysis and promoter modelling as bioinformatics tools for the identification of target genes from expression array data. Pharmacogenomics 2:25–36

Xu CS, Zhao LF, Yang KJ, Zhang JB (2004) The origin and the function of liver stem cell. Acta Biologiae Exp Sinica 37:72–77

Xu CS, Chang CF, Yuan JY, Li WQ, Han HP, Yang KJ, Zhao LF, Li YC, Zhang HY, Rahman S, Zhang JB (2005) Expressed genes in regenerating rat liver after partial hepatectomy. World J Gastroenterol 11:2932–2940

Xu CS, Chang CF (2007) Expression profiles of the genes associated with metabolism and transport of amino acids and their derivatives in rat liver regeneration. Amino acids. doi:10.1007/s00726-00007-00576-00722

Yue H, Eastman PS, Wang BB, Minor J, Doctolero MH, Nuttall RL, Stack R, Becker JW, Montgomery JR, Vainer M, Johnston R (2001) An evaluation of the performance of cDNA microarrays for detecting changes in global mRNA expression. Nucleic Acids Res 29:41–51

Zhang CT, Chou KC (1993) Graphic analysis of codon usage strategy in 1490 human proteins. J Protein Chem 12:329–335

Zhang CT, Chou KC (1994) Analysis of codon usage in 1562 E. Coli protein coding sequences. J Mol Biol 238:1–8

Zhang CT, Chou KC (1996) An analysis of base frequencies in the anti-sense strands corresponding to the 180 human protein coding sequences. Amino Acids 10:253–262

Zhang S, Kim KH (1996) Acetyl-coA carboxylase is essential for nutrient-induced insulin secretion. Biochem Biophys Res Commun 229:701–705