Effects of the pathogen infection-related genes on rat liver regeneration following 2/3 hepatectomy☆

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Received December 17, 2006

Abstract

Objective. The paper intends to discuss the roles of the genes associated with pathogenic bacteria, fungi, parasite infection during liver regeneration (LR) at transcriptional level. *Methods.* The genes associated with pathogen infection were obtained by collecting the data of databases and referring to thesis, and the gene expression changes during rat LR were checked by the Rat Genome 230 2.0 Array. *Results.* It was found that 83 genes were associated with LR. The initial and total expressing gene numbers occurring in initiation of LR (0.5 – 4 hours after PH), transition from G0 to G1 (4 – 6 hours after PH), cell proliferation (6 – 66 hours after PH), cell differentiation and reorganization of structure-function (72 – 168 hours after PH) were 42, 13, 37, 3 and 43, 32, 77, 83, respectively, illustrating that genes associated with regeneration were mainly triggered at the early stage of LR (0.5 – 4 hours after PH), and worked at different phases. The total times of their up and down-regulated expression were 347 and 157, demonstrating that expression of the majority of genes was enhanced in LR, while the minority attenuated. According to the gene expression changes, their expression patterns could be classified into 19 types, indicating the cellular physiological and biochemical activities during LR were diverse and complicated. *Conclusion.* The capability of regenerating liver to contradict the infection of pathogen is improved, and 83 genes associated with LR play an important role. [Life Science Journal. 2007; 4(2): 50 – 55] (ISSN: 1097 – 8135).

Keywords: partial hepatectomy; Rat Genome 230 2.0 Array; pathogen infection; genes; liver regeneration

1 Introduction

Liver has strong capacity for regeneration[1]. After partial hepatectomy (PH) or chemical damage[2], remnant hepatocytes carry a course including activation[3,4], differentiation[5], proliferation[6], redifferentiation and structure-function rebuild to compensate the lost liver tissue[7,8], calling liver regeneration (LR).

Liver is susceptible to infection of bacteria, fungi and parasite etc[9]. Infective liver diseases in body happen because of destruction of host cytoskeleton and interference of the signal conduction[10]. Study indicates that more than 160 genes are associated with the infection, also including interactions among gene-gene. It is hardly possible to clarify the action of genes associated with pathogen infection during LR unless high-throughput gene expression analysis is used[11,12]. So, we used the Rat Genome 230 2.0 Array containing 160 genes associated with pathogen infection detect gene expression changes after PH. 83 genes were identified associated with LR. Meanwhile, their expression character, patterns and actions during LR were primarily analyzed.

2 Materials and Methods

2.1 Regenerating liver preparation

Healthy Sprague-Dawley rats weighing 200 – 250 g were obtained from the Animal Center of Henan Normal University. The rats were separated into two groups at random and each group included 6 rats (male: female = 1:1). Partial hepatectomy (PH) was performed according to Higgins and Anderson[13], the left and middle lobes of liver were removed. Rats were killed by cervical verte-

☆Supported by the National Natural Science Foundation of China, No. 30270673.

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bra dislocation at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 54, 66, 72, 120, 144 and 168 hours after PH and the regenerating livers were observed at corresponding time point. The livers were rinsed three times in PBS at 4ºC, then 100 – 200 mg livers from middle parts of right lobe of each animal (0.1 – 0.2 g × 6, in each group) were gathered and mixed together, then stored at –80ºC. The sham-operation (SO) groups were the same with PH ones except the liver lobes were not removed. The laws of animal protection of China were enforced strictly.

2.2 RNA isolation and purification

Total RNA was isolated from the 1 – 2 g frozen livers according to the manual of Trizol kit (Invitrogen Corporation, Carlsbad, California, USA)[14] and then purified based on the guide of RNeasy mini kit (Qiagen, Inc, Valencia, CA, USA)[15]. The total RNA integrity was confirmed by agarose electrophoresis by checking the ratio of 28S rRNA to 18S rRNA. Total RNA concentration and purification were estimated by optical density measurements at 260/280 nm[16].

2.3 cDNA, cRNA synthesis and purification

As template, 1 – 8 μg total RNA was used for cDNA synthesis. cDNA purification was based on the way established by Affymetrix[17]. cRNA labeled with biotin was synthesized using cDNA as the template and then purified[17]. Measurement of cDNA, cRNA concentration and purification were the same as above.

2.4 cRNA fragmentation and microarray detection

15 μl (1 μg/μl) cRNA incubated with 5 × fragmentation buffer at 94ºC for 35 minutes was digested into 35 – 200 bp fragments. Rat Genome 230 2.0 microarray produced by Affymetrix was prehybridized, then the hybridization buffer added at 45ºC, 60 rpm for 16 hours. The microarray was washed and stained by GeneChip fluidics station 450 (Affymetrix Inc, USA). The chips were scanned by GeneChip Scan 3000 (Affymetrix Inc, USA), and the signal values of gene expression were observed[18].

2.5 Microarray data analysis

Signal values were quantified and normalized by GeneChip Operating Software (GCOS) 1.2. Quantified signal intensities were obtained by deducting foreground signal values. Signal intensities were replaced by 200 when they were < 200. When experiment/control (Ri) was between 0.1 and 10, Ri was taken as natural logarithms to generate lnRi and the normalize coefficient factor (ND) was taken by averaged Ri. The modified signal values were generated by ND multiplying control, and were replaced by 200 when it was < 200[18].

2.6 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 analysis were conducted on these values with GeneMath, GeneSpring (Silicon Genetics, San Carlos, CA) and Microsoft Excel Software (Microsoft, Redmond, WA)[18].

2.7 Identification of genes associated with a biological process

Firstly, the curated annotations describing pathogen infection was adopted from the GENEONTOLOGY database (www.geneontology.org/), and input into the databases at NCBI (www.ncbi.nlm.nih.gov/) and RGD (rgd.mcw.edu/) to identify the rat, mouse and human genes associated with the specific biological process. And we collated the influential genes according to the biological pathway maps embodied by GENMAPP (www.genmapp.org), KEGG (www.genome.jp/kegg/pathway.html#amino) and BIOCARTA (www.biocarta.com/genes/index.asp). The genes associated with the biological process were cross-checked 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 more than two-fold change in the rat regenerating livers, were referred to as rat homologous genes. The genes that displayed reproducible results with the three independent analysis with the Rat Genome 230 2.0 Array, that revealed more than two-fold change in expression at least at one time point, and that showed a significant difference ($P \leq 0.05$) or an extremely significant difference ($P \leq 0.01$) between PH and SO, were included as being associated with LR.

3 Results

3.1 General statement of expression of the pathogen infection-associated genes during LR

According to the data from NCBI, GENMAPP, KEGG, BIOCARTA and RGD databases, 155, 23 and 239 genes were associated with pathogenic bacteria, fungi and parasite respectively, in which 101, 5 and 133 genes were contained in Rat Genome 230 2.0 Array separately. Among them, corresponding 50, 1 and 66 genes revealed meaningful changes in expression at least at one time point

after PH showed significant or extremely significant differences in expression when comparing PH with SO, and were repeated three times of detection by Rat Genome 230 2.0 Array, suggesting that the genes were associated with LR. The analysis indicated that 40 genes were up, 20 genes down, and 23 genes up in some time points and down in others (Abbr. up/down-regulation) in regenerating liver. The range of up-regulation was from 2 to 128 times higher than control, and that of down-regulation was 2 – 12.5 fold (Table 1).

Table 1. Expression abundance of 83 genes associated with pathogen infection during LR

Name	Abbr.	Assoicated to others	Fold difference	Name	Abbr.	Assoicated to others	Fold difference
1 Bacteria	Akt1	3	3.9	Collagen type XVIII alpha 1	Col18a1		3.1
V-akt murine thymoma viral oncogene homolog 1	*Casp1	3	3.0	complement receptor 2	Cr2		6.0
caspase 1	*Ccl2	3	128.0	connective tissue growth factor	Ctgf		13.9
chemokine ligand 2	*Ccl20		8.0	Cytochrome b-245 beta polypeptide	Cybb	1	2.5
Chemokine ligand 20	Mif	3	3.2	FMS-like tyrosine kinase 1	Flt1		2.3
macrophage migration inhibitory factor	Myd88	3	2.1	group specific component	Gc		3.4
myeloid differentiation primary response gene 88	Cybb	3	2.5	glutathione reductase	Gsr		11.3
Cytochrome b-245 beta polypeptide	Defa	2	4.8	hemoglobin beta chain complex	*Hbb		2.5
defensin alpha	Fcgr1		2.6	Hook homolog 3	Hook3	1	5.8
Fc receptor IgG high affinity I	Hook3	3	5.8	heat shock 27kDa protein 1	Hspb1	1	11.0
hook homolog 3	Irf3	3	2.6	intercellular adhesion molecule 1	Icam1		3.0
interferon regulatory factor 3	Hspb1	3	11.0	interferon gamma	Ifng	1	6.5
heat shock 27kDa protein 1	Ifna1		13.0	interleukin 1 receptor antagonist	Il1rn		16.3
interferon-alpha 1	Ifng	3	6.5	interleukin 5	Il5		3.5
interferon gamma	Ifngr2		2.7	interferon regulatory factor 3	Irf3		2.6
interferon gamma receptor 2	Masp1	3	3.0	Janus kinase 2	Jak2		6.5
mannan-binding lectin serine peptidase 1	Nfkbia		6.8	keratin complex 2 basic gene 8	Krt2-8		4.0
nuclear factor of kappa light chain gene enhancer in B-cells inhibitor alpha	Pla2g2a	3	11.3	Lectin mannose-binding 1	Lman1		2.0
phospholipase A2 group IIA	Ppm1d		6.8	mannan-binding lectin serine peptidase 1	Masp1	1	3.0
protein phosphatase 1D magnesium-dependent delta isoform	Prkcb1	3	8.2	macrophage migration inhibitory factor	Mif	1	3.2
Protein kinase C beta 1	Tlr2	3	10.6	myeloid differentiation primary response gene 88	Myd88	1	2.1
toll-like receptor 2	Tnf	3	3.2	pyruvate kinase liver and RBC	Pklr		6.6
tumor necrosis factor	Cdh1	3	0.4	phospholipase A2 group IIA	Pla2g2a	1	11.3
cadherin 1	Cebpe		0.1	Protein kinase C beta 1	Prkcb1	1	8.2
CCAAT/enhancer binding protein epsilon	Chit1	3	0.1	Transferrin	Tf		2.7
chitinase 1	Cst11		0.1	transforming growth factor beta 1	Tgfb1		4.0
cystatin 11	Ltbr	3	0.4	toll-like receptor 2	Tlr2	1	10.6
lymphotoxin B receptor	Mbl2	3	0.2	Heat shock 70kDa protein 1B	Hspa1a	1	0.2
mannose-binding protein C	Il15	3	0.4	inhibitor of kappa B kinase beta	Ikbkb		0.3
interleukin 15	Hspa1a	3	0.2	colony stimulating factor 2	Csf2		0.3
Heat shock 70kD protein 1B	Plunc		0.3	interleukin 15	Il15	1	0.4
palate, lung, and nasal epothelium carcinoma associated	Prf1	3	0.2	interleukin 1 beta	*Il1b		0.4
perforin	Prq2		0.3	integrin beta 2	Itgb2		0.5
proteoglycan 2 bone marrow	Tcrb	3	0.2	lymphotoxin B receptor	Ltbr	1	0.4
T-cell receptor beta chain	Psen2	3	0.2	mannose-binding protein C	Mbl2	1	0.2
presenilin 1	Tlr4	3	0.5	perforin 1	Prf1	1	0.2
toll-like receptor 4	Wasl		6.8, 0.5	presenilin 2	Psen2	1	0.2
Wiskott-Aldrich syndrom-like	Ptqs2	3	2.1, 0.1	cd36 antigen	Cd36		0.1
prostaglandin-endoperoxide synthase 2	Stab1		6.5, 0.4	cadherin 1	Cdh1	1	0.4
stabilin 1	Prdx3	3	2.6, 0.5	chitinase 1	Chit1	1	0.1
Peroxiredoxin 3	Ppbp		2.1, 0.1	T-cell receptor beta chain	Tcrb	1	0.2
pro-platelet basic protein	Defb1	3	2.5, 0.4	toll-like receptor 4	Tlr4	1	0.5
defensin beta 1	Ela2	3	52.0, 0.5	apolipoprotein E	Apoe		0.1
elastase 2	Il2	3	3.5, 0.3	cathelicidin	Cramp	1	2.8, 0.4
interleukin 2	Il6	3	6.1, 0.3	defensin beta 1	Defb 1	1	2.5, 0.4
interleukin 6	Indo	3	2.3, 0.4	Duffy blood group chemokine receptor	Dfy		8.5, 0.4
indoleamine 2,3-dioxygenase	Cramp	3	2.8, 0.4	elastase 2	Ela2	1	52.0, 0.5
cathelicidin	Ncf1		3.7, 0.2	coagulation factor 3	F3		2.0, 0.1
neutrophil cytosolic factor 1	Nfkb1		2.3, 0.4	glycophorin C	Gypc		2.1, 0.5
nuclear factor of kappa light chain gene enhancer in B-cells 1	Lyz		3.7, 0.4	hemoglobin epsilon 1	Hbe1		2.5, 0.1
lysozyme				interleukin 13 receptor alpha 2	Il13ra2		4.3, 0.4
2 Fungi	Defa	1	5.0	interleukin 2	Il2	1	3.5, 0.3
defensin alpha				interleukin 4	Il4		2.6, 0.1
3 Parasite	Actl6		2.3	interleukin 6	Il6	1	6.1, 0.3
actin-like 6	Akt1	1	3.9	indoleamine 2,3-dioxygenase	Indo	1	2.3, 0.4
V-akt murine thymoma viral oncogene homolog 1	Tnf	1	3.2	Peroxiredoxin 3	Prdx3	1	2.6, 0.5
tumor necrosis factor	Capg		2.5	prostaglandin-endoperoxide synthase 2	Ptgs2	1	2.1, 0.1
capping protein gelsolin-like	*Casp1	1	3.0	solute carrier family 13 alpha member 4	Slc13a4		2.0, 0.2
caspase 1	*Ccl2	1	128.0	similar to polycystic kidney disease 2	Spp1		2.7, 0.5
chemokine ligand 2				signal transducer and activator of transcription 4	Stat4		4.0, 0.1

* Reported genes associated with LR.

3.2 Expression changes of the pathogen infection-associated during LR

At each time point of LR, the numbers of initially up, down-regulated and totally up, down-regulated gene were in sequence: both 11 and 4 at 0.5 hours; 11, 2 and 20, 3 at 1 hours; 6, 1 and 19, 2 at 2 hours; 6, 2 and 22, 3 at 4 hours; 3, 2 and 15, 7 at 6 hours; 1, 1 and 14, 7 at 8 hours; 1, 0 and 11, 5 at 12 hours; 5, 5 and 19, 9 at 16 hours; 3, 5 and 22, 12 at 18 hours; 0, 1 and 16, 11 at 24 hours; 2, 0 and 11, 6 at 30 hours; 0, 0 and 15, 13 at 36 hours; 1, 1 and 13, 4 at 42 hours; 4, 0 and 27, 11 at 48 hours; 0, 1 and 14, 10 at 54 hours; 0, 1 and 12, 9 at 60 hours; 0, 0 and 16, 6 at 66 hours; 0, 0 and 9, 7 at 72 hours; 1, 0 and 15, 4 at 96 hours; 2, 0 and 16, 11 at 120 hours; 0, 0 and 17, 9 at 144 hours; 0, 0 and 13, 4 at 168 hours. As far as the initial expression of the above 83 genes was concerned, 57 and 26 genes were initially up-regulated and down-regulated during LR, respectively. A detailed introduction is as follows: at the initiation stage (0.5 – 4 hours after PH), the G0/G1 transition phase (4 – 6 hours after PH), cell proliferation period (6 – 66 hours after PH), cell differentiation and the structure-function reorganization stage of LR (72 – 168 hours after PH), the number of initially up and initially down-regulated genes were 34 and 9, 9 and 4, 20 and 17, 3 and 0. The whole situation of the genes expression was that the total frequencies of up and down-regulated expression were respectively 347 and 157. Specifically, at the above-mentioned four phases of LR, the number of times of up-regulation and down-regulation was separately 72 and 12, 37 and 10, 205 and 110, 70 and 35 (Figure 1).

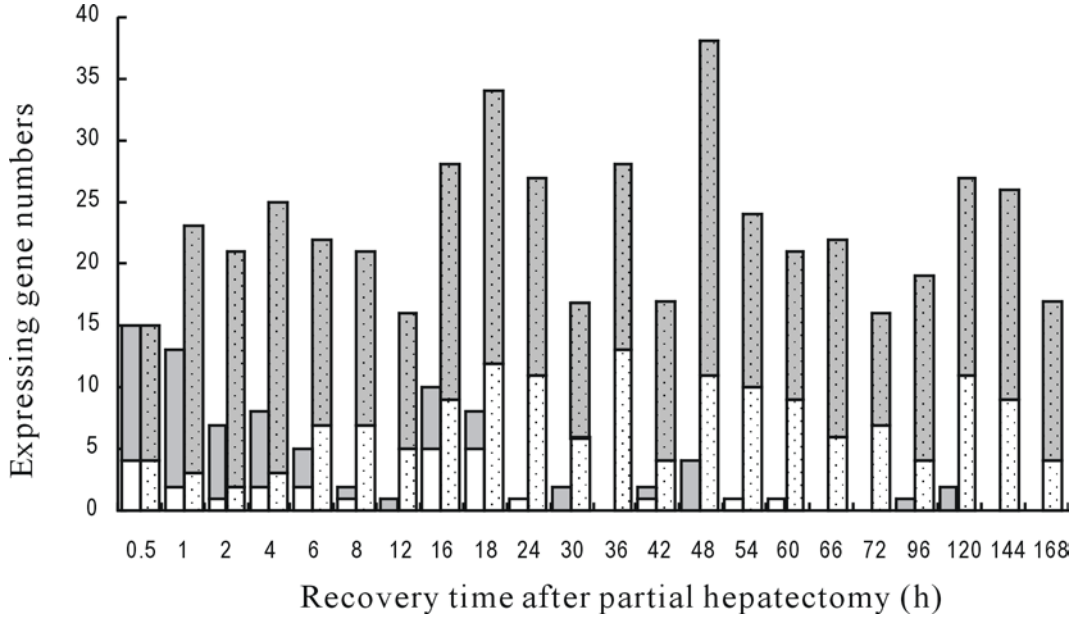

Figure 1. The initial and total expression profiles of 83 genes associated with pathogen infection at each time point of LR. Blank bars: Initially expressing gene number; Dotted bars: Total expressing gene number; Grey-background bars: Up-regulated genes; White-background bars: Down-regulated genes. Expression change of the genes spans the whole live regeneration. Initially up-regulated genes are predominate at 0.5 – 6, 12, 30 – 48 and 96 – 120 hours after PH; initially down-regulated genes are overwhelm at 24 and 54 – 60 hours; there are no initially expressed genes at 36, 66 – 72 and 144 – 168 hours.

3.3 The expression patterns of the pathogen infection-associated during LR

A total of 83 genes during LR might be categorized into 19 clusters according to the changes in expression: (1) up-regulation at one time point, i.e. 1, 4, 48, 96 or 120 hours after PH (Figure 2A), involved in 8 genes; (2) up at two time points, i.e. 8 and 96 hours, 16 and 42 hours, 30 and 42 hours, 48 and 66 hours, 48 and 120 hours (Figure 2B), in 5 genes; (3) up at three time points (Figure 2B), in 2 genes; (4) up at three phases (Figure 2C), in 2 genes; (5) up at one time point/one phase, i.e. 6 and 66 – 72 hours, 18 and 48 – 60 hours, 42 and 120 –168 hours, 48 and 2 – 24 hours (Figure 2C), in 4 genes; (6) up at one time point/two phases (Figure 2D), in 4 genes; (7) up at two time points/two phases (Figure 2D), in 3 genes; (8) up at two time points/three phases (Figure 2D), in 2 genes; (9) up at more time points or phases (Figure 2E), in 10 genes; (10) down at one time point, i.e. 16 or 60 hours (Figure 2F), in 3 genes; (11) down at two time points, i.e. 18 and 54 hours, 42 and 60 hours (Figure 2F), in 2 genes; (12)

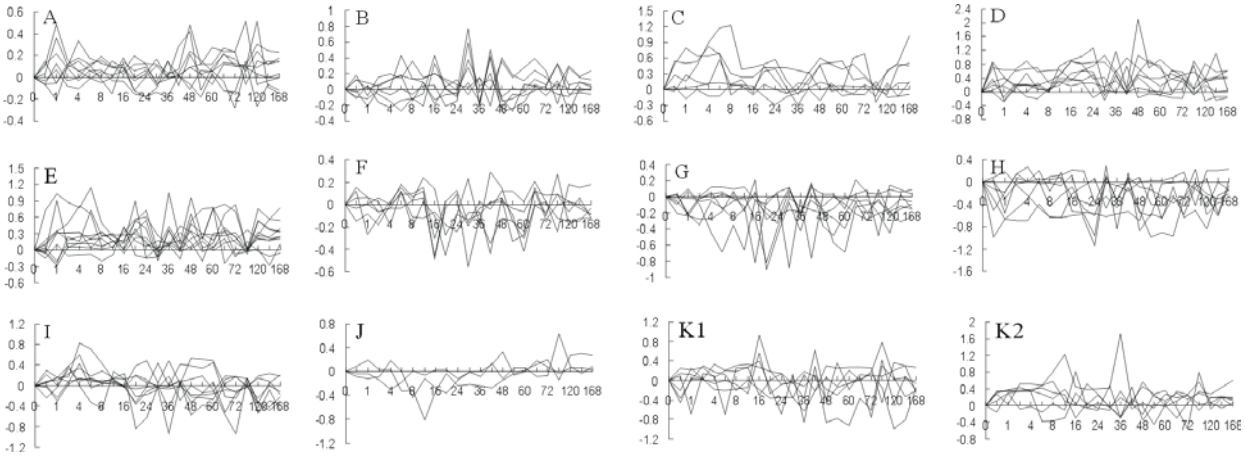

Figure 2. Expression patterns of 83 genes associated with pathogen infection during LR. A – E: Up-regulated genes; F – H: Down genes; I – K: Up/down mixed genes. X-axis represents recovery time after PH (h), Y-axis shows logarithm ratio of the signal values of genes at each time point to control.

down at four time points (Figure 2G), in 2 genes; (13) down at one phase, i.e. 4 – 12 hours, 54 – 60 hours (Figure 2G), in 2 genes; (14) down at one time point/one phase, i.e. 24 and 48 – 54 hours, 36 and 18 – 24 hours, 120 and 18 – 24 hours (Figure 2G), in 3 genes; (15) down at two time points/two phases (Figure 2H), in 3 genes; (16) down at more time points or phases (Figure 2H), in 5 genes; (17) first up and then down (Figure 2I), in 8 genes; (18) first down and then up (Figure 2J), in 3 genes; (19) up/down mixed (Figure 2K1, 2K2), in 12 genes.

4 Discussion

The roles of the genes associated with pathogen infection during LR are studied in this paper. Among 101 genes associated with pathogenic bacteria infection, *itgb2* can promote leucocyte conglutination and phagocytosis[19]. *hook3* and *lyz* can sterilize[20,21]. Five genes including *defb1* can enhance the anti-microbial capability of body[22]. Five genes including *cramp* restrain the activity of bacteria[23]. Five genes including *ccl2* activate macrophages, prevent bacterial infection[24]. Three genes including *mif* accelerate inflammation[25]. Thirteen genes including *defa5* activate immune response[26]. Five genes including *il15* promote cell activation and proliferation[27]. Four genes including *prf1* facilitate pathogen infect lymphocyte by destroying immune competent cell[28]. All of genes mentioned above reveal significant changes in expression, moreover they show same or similar changes at some time points and different at others, they together enhance regenerating liver capability for anti-pathogen and regulating inflammation. In which 50 genes associated with LR play a significant role.

Among 134 genes associated with pathogenic parasite infection, seven genes containing *il1b* contradict the infection of Plasmodium[29]. *pklr* restrains infection of vermin as Plasmodium[30]. *csf2* and TNF-α play the role in anti-microbial response after Schistosome infects[31]. *cd36* and *gypc* are involved in Plasmodium infection[32,33]. *ikbkb* promotes pathogen infection by inhibiting the activity of NF-κB[34]. *actl6* regulates transcription of immunity-associated genes post-infection[35]. *cr2* induces immune response[36]. *darc* accelerates leucocyte accumulation[37]. *il13ra2* and *ilrn* repress immunity response[38,39]. Three genes including *il5* protect against liver injury[40]. *tf* speeds phagosome circulation[41]. *ctgf* boosts chemotactic factor expression and wound repair[42, 43]. *f3* has the role of anticoagulation[44]. These genes all reveal the significant changes in expression that are same at some time points and different at others, perhaps that they together enhance regenerating liver capability for contradict pathogen and immuno-regulation activity. In which 66 genes associated with LR are essential.

In summary, some genes associated with pathogen infection are enhanced, others weakened. They together prevent regenerating liver from infecting and promote LR. In future Northern blotting, protein array, RNA interference etc. will be used to further analyze the above result at cell level.

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