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The Role of Kupffer Cells in Rat Liver Regeneration Revealed by Cell-Specific Microarray Analysis

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ABSTRACT

Liver regeneration after partial hepatectomy is a process with various types of cells involved. The role of Kupffer cells (KCs) in liver regeneration is still controversial. In this study we isolated KCs from regenerating liver and conducted cell-specific microarray analysis. The results demonstrated that the controversial role of KCs in liver regeneration could be explained with the expression patterns of TGF-α, IL-6, TNF, and possibly IL-18 during liver regeneration. IL-18 may play an important role in negative regulation of liver regeneration. The functional profiles of gene expression in KCs also indicated that KC signaling might play a negative role in cell proliferation: signaling genes were down regulated before cell division. Immune response genes in KCs were also down regulated during liver regeneration, demonstrating similar expression profiles to that of hepatocytes. The expression patterns of key genes in these functional categories were consistent with the temporal functional profiles. J. Cell. Biochem. 113: 229–237, 2012. © 2011 Wiley Periodicals, Inc.

KEY WORDS: LIVER REGENERATION; MICROARRAY; KUPFFER CELLS; IL-18; GENE FUNCTIONAL PROFILE

Liver regeneration is an orchestrated response to a loss of hepatic tissue, with various types of cells in the liver involved in the process. Kupffer cells (KCs), the resident liver macrophages, represent about 35% of the non-parenchymal liver cells in normal liver and 80–90% of the tissue macrophages present in the body [Bilzer et al., 2006]. They reside within the lumen of the liver sinusoids, adherent to the endothelial cells that compose the blood vessel walls. Together with natural killer cells, dendritic cells, and soluble compounds such as complement factors and acute phase proteins, KCs represent an important component of innate immunity.

Kupffer cells are well known to release TNF-α and IL-6 for the priming of hepatocyte proliferation in vivo in liver regeneration after partial hepatectomy (PH) [Michalopoulos, 1997, 2007; Fausto, 2000; Fausto et al., 2006]. However, the role of KCs in liver regeneration is debated because the results of experiments in KC-depleted mice are controversial [Kandilis et al., 2010]. It has been shown that KC depletion prior to PH enhances rather than depresses liver regeneration, whereas in other reports the process is delayed [Boulton et al., 1998; Takeishi et al., 1999]. Boulton et al. [1998] showed that selective KC depletion enhanced not only liver regeneration after PH but also the proliferation of hepatocytes in resting intact liver. It was hypothesized that KC depletion resulted in a reduced influence of negative hepatocyte growth regulator IL-1 after PH. On the other hand, Takeishi et al., [1999] showed that liver regeneration was delayed and the number of HGF-expressing cells was decreased in the regenerating liver after KC depletion. Both studies were based on the selective depletion of KCs by injection of liposome-encapsulated dichloromethylene diphosphonate (Cl_2MBP).

Microarray is a powerful tool to study genome wide expression pattern of tens of thousands of genes simultaneously, wherefore the expression changes of a large number of genes activated or suppressed under various biological conditions can be monitored. Microarray has been used to study rat liver regeneration after PH [Fukuhara et al., 2003; Otu et al., 2007; Xu et al., 2009]. The up- or down-regulated genes were clustered based on the expression patterns and the functions of some genes were analyzed. However, this kind of analysis is trivial, descriptive, and misleading. The global perspective of gene functions is dismissed. Moreover, the liver is composed of various differentiated cell types that play different roles during liver regeneration, and cell-specific gene expressions of liver cells during liver regeneration need to be studied.

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In order to solve the discrepancy of the KC depletion experiments and elucidate the role of KCs in liver regeneration, we isolated KCs from regenerating liver after PH and performed cell-specific microarray analysis. We used new statistic *Change P*-value provided by Affymetrix software [Affymetrix Manual, 2008] to identify differentially expressed genes. This method is more sensitive than conventional fold change method. Our results indicated that the discrepancy was due to the difference of the time for KC depletion, and the role of KCs in liver regeneration might be different in the early priming phase and in late proliferation phase.

MATERIALS AND METHODS

RAT 2/3 PARTIAL HEPATECTOMY

Healthy 12-week-old Sprague-Dawley (SD) rats, 230 ± 20 g, were obtained from the Experimental Animal Center of Henan Normal University, Xinxiang, Henan, China. The animal experiments were conducted in strict compliance with animal welfare regulations approved by Institutional Animal Care and Use Committee of Henan Normal University in China. The rats were bred in $21 \pm 2^{\circ}$C, relative humidity $60 \pm 10\%$, illumination time 12 h/day (8:00–20:00), and free access to water and food. In the experiment, a total of 114 rats were randomly divided into 19 groups, and 6 rats in each. Among these rats, 9 groups, total 54 rats for 2/3 PH, 9 groups, total 54 rats for sham operation (SO), and 1 group, total 6 rats for the control. PH was performed on the rats according to the procedure originally described by Higgins and Anderson [1931]. For SO, surgical operation of rats was done as did for the PH, but no liver lobes dissection. After that, the rats were bred in above-mentioned conditions, and their regenerating livers were taken for isolating liver cells, respectively, at 0, 2, 6, 12, 24, 30, 36, 72, 120, and 168 h after PH. Zero hour means that when the liver lobes were removed, the remaining liver was immediately used for cell isolation.

ISOLATION OF LIVER CELLS

At different time points, the recovering rats were recruited. The rats were anesthetized by aether and sterilized with 75% alcohol, and the upper abdomen of the rat was opened to expose liver. The liver was turned over to expose the hepatic portal vein. After a tube was inserted into the hepatic portal vein, D-Hank's solution at 37°C was perfused into the liver at the rate of 10–20 ml/min [Selgen, 1976]. When the liver surface turned slight yellow, 30 ml 0.05% collagenase IV replaced D-Hank's solution to perfuse continuously into the liver at the rate of 1–2 ml/min. The perfused liver was removed and incubated in a flask with 0.05% collagenase IV at 37°C for 15 min. Then 4°C PBS was added and the liver was combed with glass needle to disperse cells. The dispersed cells were collected and filtered through 400-mesh nylon net. The cells were suspended in cold PBS buffer. Cell concentration was adjusted to 1×10^8 cells/ml for use.

ISOLATION OF KUPFFER CELLS

The above cell suspension 6 ml were slowly spread out onto 10 ml centrifuge tube, which contained 2 ml 90% percoll bellow, and 2 ml 32% percoll upper. It was centrifugated at 200 g for 5 min. The supernatant was diluted twice with equal volume PBS and centrifugated at 400 g at 4°C for 2×2 min. The harvested sediment was non-parenchymal cells (NPCs) [Blair et al., 1995]. The cells were suspended and mixed with 10 μl/ml rat anti-CD68-polyethylene (CD68-PE) antibody and incubated for 15 min at 4°C. Then 10 μl/ml rat anti-PE magnetic beads were added, and incubated for 15 min at 4°C. The complex was loaded onto the separation column and allowed to flow naturally. The column was washed twice with 4°C PBS buffer, and then the magnetic field was removed. The column was washed once again with 4°C PBS buffer. The fraction was collected, which contained KCs [Grisham, 1983]. The vitality of KCs are ≥90%, and the percentage of red blood cell numbers is ≤0.1%. The qualified samples from six rats in each group were mixed together for use.

IMMUNOCHEMICAL ANALYSIS

Ten microliters KCs suspension was spread on glass slice, and fixed with 10% formaldehyde for 30 min, then treated by microwave for 5 min. The cells were treated by 3% H_2O_2 for 5 min to eliminate exogenous peroxidase, then 2% Triton X-100 for 5 min, and rabbit serum for 15 min. After that, the slice was incubated overnight at 4 °C in fist antibody of 1:200 dilutions (v/v) of ectodermal dysplasia antigen 2 (Ed2) and lysozyme (LYZ), respectively. As the control, PBS was used to replace the first antibody. Then 1:5,000 diluted second antibody (v/v) labeled with biotin was used to incubate the slice at 37°C for 60 min. After that, the slice was incubated in streptavidin–biotin complex (SABC) at 37°C for 30 min. The slice was stained with 3,3′-diaminobenzidine (DAB), and re-stained with hematoxylin. After dehydration and vitrification, the slice was observed under the microscope.

RNA EXTRACTION AND MICROARRAY ANALYSIS

1×10^6 isolated cells were taken, and their total RNA was extracted according to the manual of Trizol reagent (Invitrogen Corporation, Carlsbad, California) [Norton, 1992], and purified following the RNeasy mini protocol (Qiagen, Inc, Valencia, CA) [Scott, 1995]. The quality of total RNA was assessed by optical density measurement at 260/280 nm and agarose electrophoresis (180 V, 0.5 h). It was regarded as qualified sample when 28S RNA to 18S RNA is equal to 2:1. T7-oligo dT(24) (W.M. Keck Foundation, New Haven, CT) SuperScript II RT (Invitrogen Corporation) and 5 μg of total RNA was used to synthesize the first strand of cDNA. The second strand synthesis was performed using the Affymetrix cDNA single-stranded cDNA synthesis kit. The cDNA product was purified following the cDNA purify protocol. The 12 μl purified cDNA and the reagents from the GeneChip In Vitro Transcript Labeling Kit (ENZO Biochemical, New York, NY) were used to synthesize biotin-labeled cRNA. The labeled cRNA was purified using the RNeasy Mini Kit columns (Qiagen). Their concentration, purity and quality were assessed as above "RNA extraction." Fifteen microliters 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 Array was put into a hybridization buffer, which was prepared following the Affymetrix protocol [Affymetrix, 2002], and hybridized in a rotating chamber (60 rpm, 16 h, 45°C). The hybridized arrays were washed by wash buffer to remove the hybridization buffer, and stained in

GeneChip fluidics station 450 (Affymetrix Inc., Santa Clara, CA). Then the arrays were scanned and imaged with a GeneChip scanner 3000 (Affymetrix Inc.) [Guo et al., 2008].

DATA ANALYSIS AND NORMALIZATION

The images were converted into normalized signal values, signal *detection P* and experiment/control *change P*-values using GCOS 1.4 software [Affymetrix Manual, 2008]. The data of each array were initially normalized through scaling all signals to a target intensity of 200. Gene expression was analyzed using the defaults parameter settings. When *detection P*-value was <0.05, it meant the gene was present (P); when *detection* $P > 0.05$, it meant the gene was marked absent (A). On the other hand, the ratio of normalized signal values of PH group to that of control were used to calculate the relative value. When *change P*-value was <0.002, it meant the gene expression was increased compared with control; $0.002 < P < 0.998$, gene expression had no change; $P > 0.998$, gene expression was decreased. To minimize errors from microarray analysis, each sample was analyzed at least three times using Rat Genome 230 2.0 Array. The averages of *P*-values were used.

The processed microarray data are available online as supporting information files. The data file is an Excel file. All transcripts expressed in hepatocytes were marked pink with the addition of *detection P*-value lower than 0.15 (average *detection* $P < 0.05$). All transcripts up regulated at different time points were marked yellow with the addition of *change P*-value lower than 0.018 (average *change* $P < 0.002$) in PH and SO group. All transcripts down regulated were marked blue with the addition of *change P*-value higher than 8.982 (average *change* $P > 0.998$).

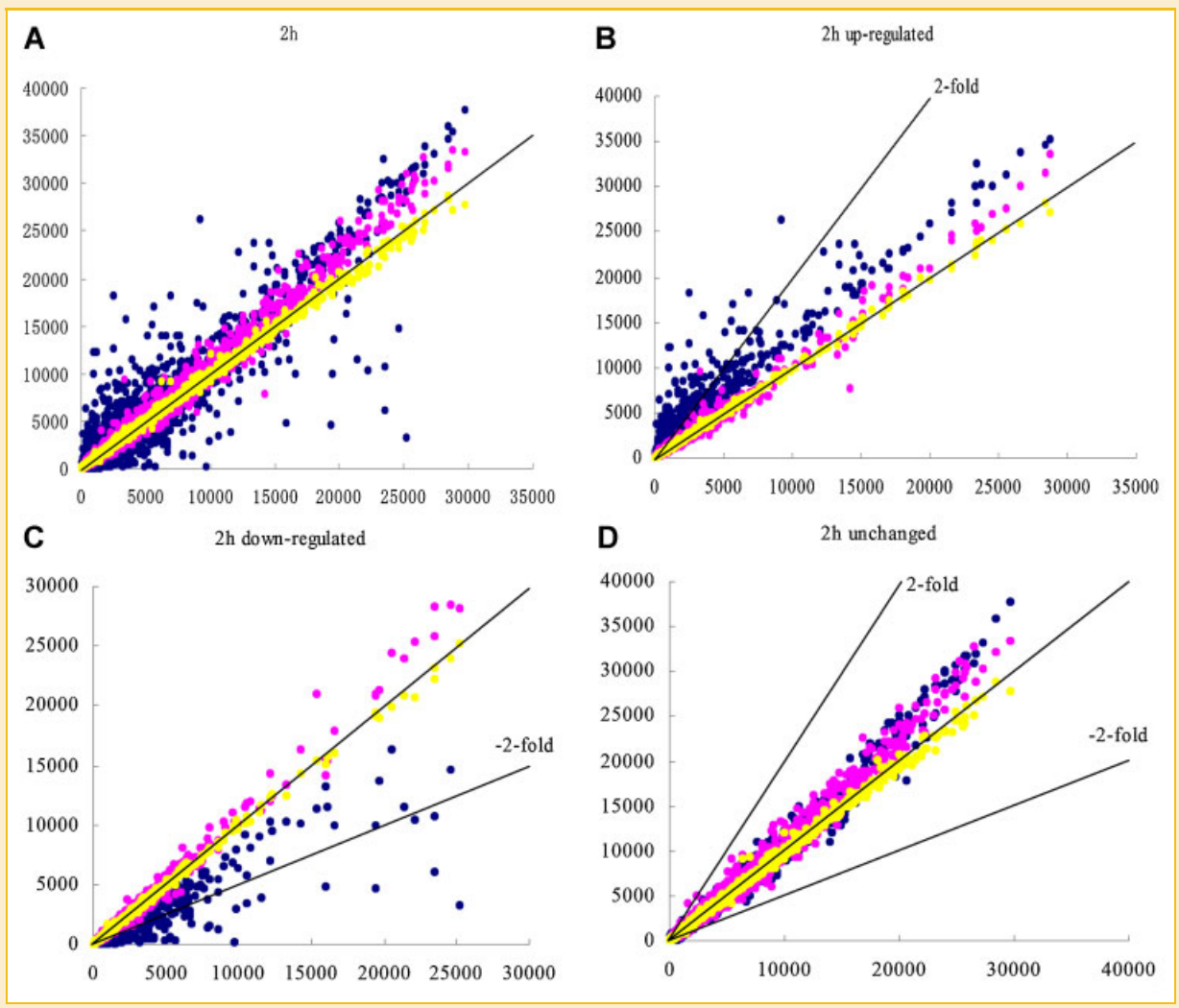

Fig. 1. A: Comparison of transcript expression profiles of PH and SO group at 2 h after PH: scatter plots of the average signal value against that of the control group (0 h). Yellow dots are from one sample of the control group (0 h). Pink dots are from SO group. Blue dots are from PH group. B–D: Comparison of *P*-value and fold change methods. B: The transcripts up-regulated at 2 h (*change* $P > 0.998$). Some blue dots are within twofold change. C: The transcripts down-regulated at 2 h (*change* $P < 0.002$). Some blue dots are within twofold change. D: The transcripts unchanged at 2 h (*change* $0.002 < P < 0.998$). Almost all blue dots are within twofold change. The results indicated *change P*-value method was more sensitive than twofold change method to identify gene expression change.

FUNCTIONAL PROFILING

The gene lists of up- and down-regulated genes at different time points after PH were obtained through above data analysis and used to conduct functional profiling.

Online automatic gene ontological analysis tool ProfCom was used to perform gene functional profiling (http://webclu.bio.wzw.tum.de/profcom/start.php). The "Operation" was set "AND". The enriched gene categories with similar functions were classified together, such as cell growth (cell proliferation, cell division, cell cycle, mitosis, DNA replication, etc.), signaling (signal transduction, cell surface receptor linked signal transduction, intercellular signaling cascade, signaling molecules, etc.), transcription (transcription factors, regulation of transcription), immune response (inflammatory response, acute-phase response, antigen representation, etc.), and others (angiogenesis, gamete generation, calcium ion binding, etc.).

REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR)

The mRNA sequences for randomly selected genes *cd86*, *icam1*, *pcna*, *trim24*, and *β-actin* were retrieved and the primers were designed for each transcript by primer express 2.0 software and synthesized in Beijing Sunbiotech Co. Ltd. RT-PCR was performed with total RNA using SYBR Green on a 214 Rotor-Gene 300 (Corbett Robotics, Valencia, CA). Threshold cycle numbers for each gene were normalized to that of *β-actin* as described earlier [Wang and Xu, 2010]. All RT-PCR experiments were done in triplicate. After Ct values were generated, relative quantity of the target genes were calculated according to the standard curves. In the general controls, water was used to replace template (NTC). In the no reverse transcription control, reverse transcription enzyme (AMV) was omitted in the RT steps.

RESULTS

IMMUNOCHEMICAL ANALYSIS

The purity of KCs is >96% as displayed by immunochemical staining of ED2 and LYZ antibody.

TRANSCRIPT EXPRESSION PATTERNS AFTER PH

The transcript expressions of PH group and SO group are different and could be easily identified (Fig. 1A). The yellow dots are the signal values of one sample of the control, which align with the diagonal very well. This indicates the three samples of the control group are consistent and repeated well. If the scanning results of the three samples were not very consistent, the dots would deviate the diagonal. The pink dots are from SO group. SO might cause changes in the transcript expression pattern of KCs due to KC's role in immune response, so the dots scatter around and deviate the

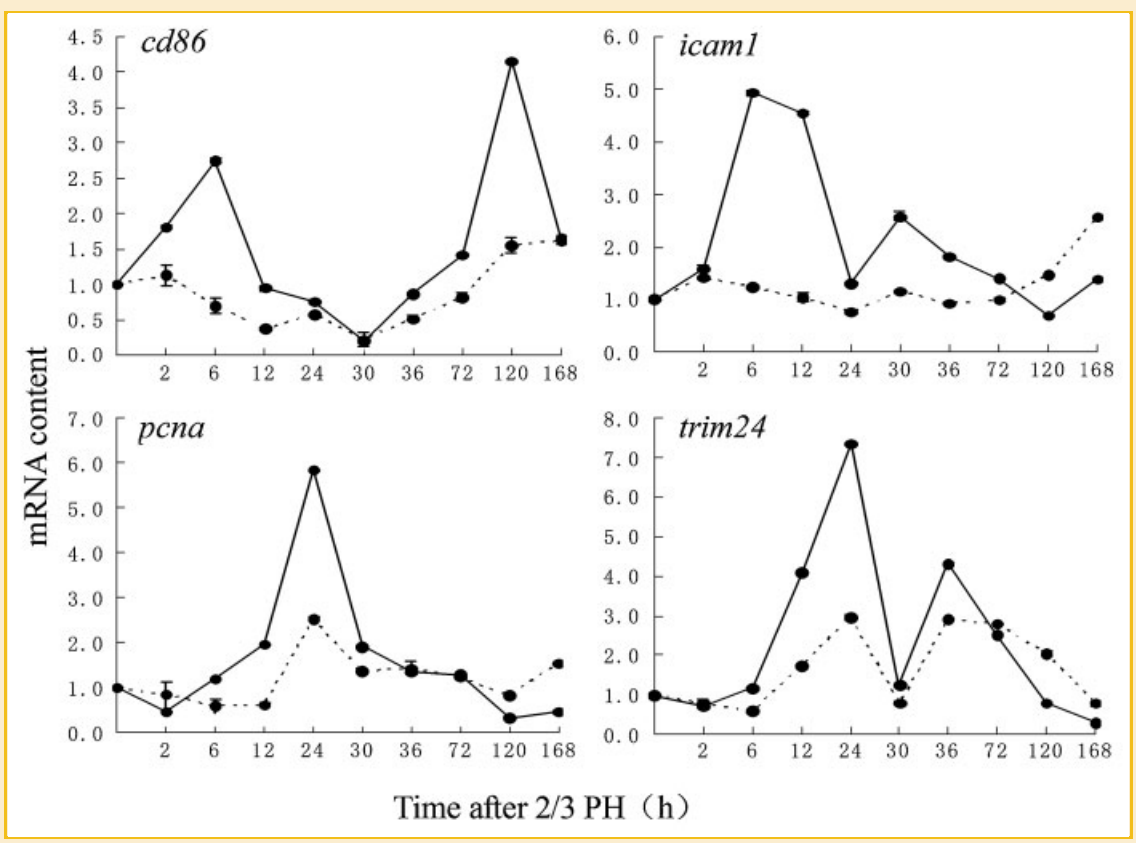

Fig. 2. The comparisons of relative mRNA content to time 0 of micaroarray and RT-PCR: the real lines denote the results of RT-PCR; the virtual lines denote the results of micrarray.

diagonal. The blue dots are from PH group, which caused huge changes in the transcript expression patterns. The dot plot of expression profile is better than any other methods to manifest the reliability of the microarray data, because if the data were not reliable, the dots from SO group would be indistinguishable from the dots from PH group. Any errors could be found in this way.

The *Change P*-value method provided by Affymetrix software GCOS 1.4 [Affymetrix Manual, 2008] to identify differentially expressed genes has not been widely used. We used *Change P*-values rather than the fold change in signal intensity to identify the up- or down-regulation of gene expression. The software automatically performed normalization before comparison. The Wilcoxon's Signed Rank test is used in comparison analysis [Affymetrix Manual, 2008]. Each probe set on the experiment array is compared to its counterpart on the baseline array, and a *Change P*-value is calculated indicating an increase, decrease, or no change in gene expression [Affymetrix Manual, 2008]. This method is theoretically better than fold change because according to Affmetrix's manual the signal intensity is almost meaningless when the *detection P*-value is not right. Usually twofold change is used to identify regulated gene expression, but the fold change of 2 is quite arbitrary. The expression changes of some signaling genes may be only little, but the effects may be great. *Change P*-value method seems to be more accurate and sensitive. More transcripts were identified as regulated by *change P*-value method than by twofold change method (Fig. 1), and most dots of unchanged transcripts by *change P*-value were within the range of twofold change.

The comparison of expression patterns of microarray and RT-PCR of randomly selected genes *cd86*, *icam1*, *pcna*, and *trim24* are shown in Figure 2. Although the expression patterns of some genes detected by RT-PCR were a little different from the microarray data, the expression trends of these genes from both techniques were generally similar.

EXPRESSION PATTERNS OF CYTOKINES AND GROWTH FACTORS

Boulton et al. [1998] proposed that the absence of IL-1β mRNA after PH in KC depleted liver was responsible for the enhancing effect of KC depletion because IL-1β is a negative hepatocyte growth regulator. However, IL-1β mRNA during 1–3 days after PH was at the same level as before PH (Fig. 3A). It is difficult to explain why liver regeneration was delayed in mice with KC depletion at 3 day post-PH.

We examined the expression patterns of almost all cytokines and growth factors. The expression patterns of IL-1β, IL-10, IL-18, TGF-α, TNF, and IL-6 are shown in Fig. 3. Among all cytokines and growth factors, only the expression patterns of IL-18, TGF-α, TNF, and IL-6 can explain the role of KC depletion. IL-18 was highly expressed in KCs in normal resting liver and the expression even increased at 2 h after PH (Fig. 3B). This can explain why KC depletion from resting intact liver with or without PH enhanced hepatocyte proliferation. This is because KC depletion eliminates the inhibitory effect of IL-18 on hepatocyte proliferation. The expression of IL-18 significantly decreased during 6–72 h after PH in rat KCs, while the expression of TGF-α, TNF, and IL-6 in KCs significantly increased in this period (24–36 h, Fig. 3C,D). It is known that TGF-α, TNF, and IL-6 all enhance hepatocyte proliferation after

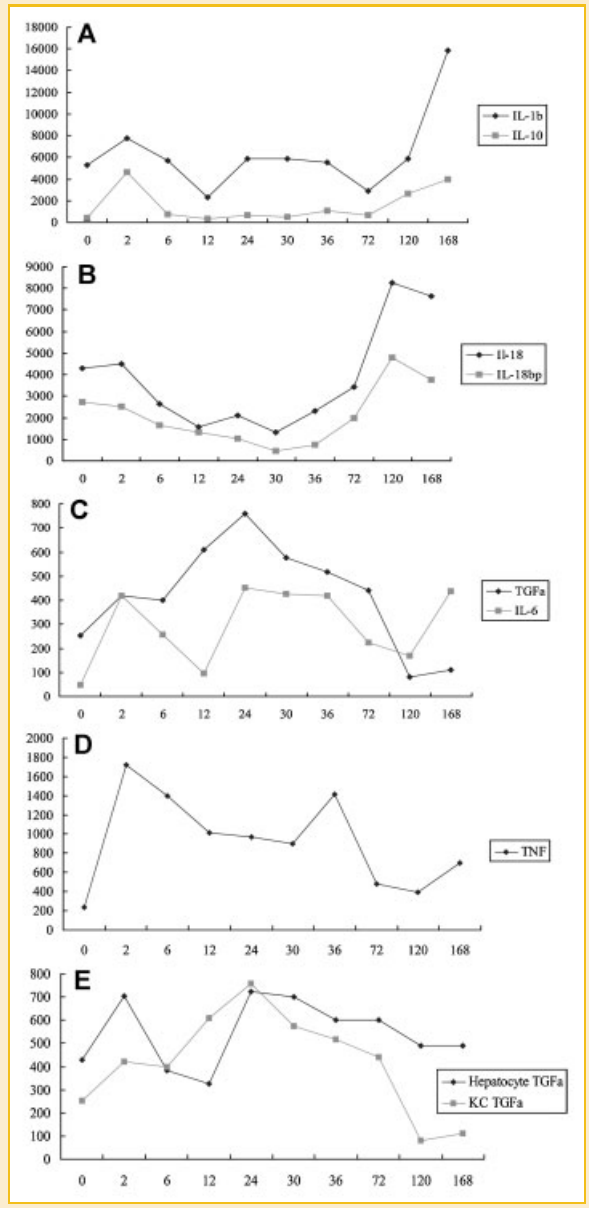

Fig. 3. The expression patterns of some signal molecules. Y-axis is the average signal value in microarray chips, X-axis is the hours after PH. A: IL-10 and IL-1b. B: IL-18 and IL-18bp. C: TGF-α and IL-6. D: TNF. E: TGF-α in hepatocytes [Jiang et al., 2011] and in KCs.

PH. Takeishi suggested KCs might control HGF production in stellate cells to regulate liver regeneration after PH [Takeishi et al., 1999]. IL-6 is known to induce expression of HGF in rat stellate cells [Kariv et al., 2003]. So KC depletion during this period (for mice, 3 days after PH) reduced TGF-α, TNF, IL-6, and HGF production in stellate cells, and led to the delay in liver regeneration. TGF-α mRNA has been reported induced in hepatocytes during liver regeneration [Mead and Faosto, 1989]. In fact, TGF-α mRNA in KCs was at the same level as in hepatocytes [Jiang et al., 2011] during 24–72 h after PH (Fig. 3E), implying KCs may be another source of TGF-α during liver regeneration.

THE FUNCTIONAL PROFILES OF GENE EXPRESSION IN KUPFFER CELLS DURING LIVER REGENERATION

We further investigated the gene expression functional profile to reveal the overall role of KCs in liver regeneration.

The up-regulated genes at different time points after PH were identified with *change* P-value < 0.002, *detection* P-value < 0.05, and the genes up-regulated in SO group were excluded. The down-regulated genes were identified with *change* P-value > 0.998, and the genes not expressed in normal KCs (*detection* P-value > 0.05) and down-regulated in SO group were excluded. The functional profiles were obtained using ProfCom [Antonov et al., 2008], a web-based gene functional profiling tool, which we found the best among tens of automatic gene ontology enrichment tools comparing with the result of our manual functional profiling [Jiang et al., 2011].

We conducted gene functional profiling based on the up- or down-regulated gene lists at each time point rather than on clustering the genes according to expression pattern change, because we did not want to trace how gene expressions changed during liver regeneration. We did not cluster genes at all. What we wanted to know was, at each time point after PH, what kinds of genes were regulated, or what were the functions of those genes regulated at each time point during liver regeneration?

The predominant functional categories in up-regulated genes during 2–12 h after PH were "response to stimulus," "metabolism," and "transport." From 24 to 168 h after PH, "cell growth" "response to stimulus," and "metabolism" genes predominated, reflecting active cell proliferation in this period (data not shown). In down-regulated genes, "transcription" and "cell growth" predominated at 2 h; "cell growth," "signaling," "immune response," and "protein phosphorylation" predominated from 6 to 12 h; "immune response," "signaling," and "metabolism" predominated from 24 to 72 h; "metabolism," "response to stimulus" and "transport" from 120 to 168 h (data not shown). Immune response is the most important function of KCs. Down-regulation of "immune response" implied KCs, like hepatocytes [Jiang et al., 2011], were partially dedifferentiated during liver regeneration.

When the functional categories were studied individually, the trend of function change can be seen more clearly. The number of "cell division" genes decreased first from 2 to 12 h before it suddenly increased from 24 h after PH, implying KCs underwent some extent of cell proliferation in normal resting liver (Fig. 4). The proliferation activity was decreased first. Because "DNA replication" genes were counted in "cell division" category, the number of up-regulated "cell division" genes reached the highest at 24 h. The numbers were still high at 168 h when liver regeneration almost finished, implying

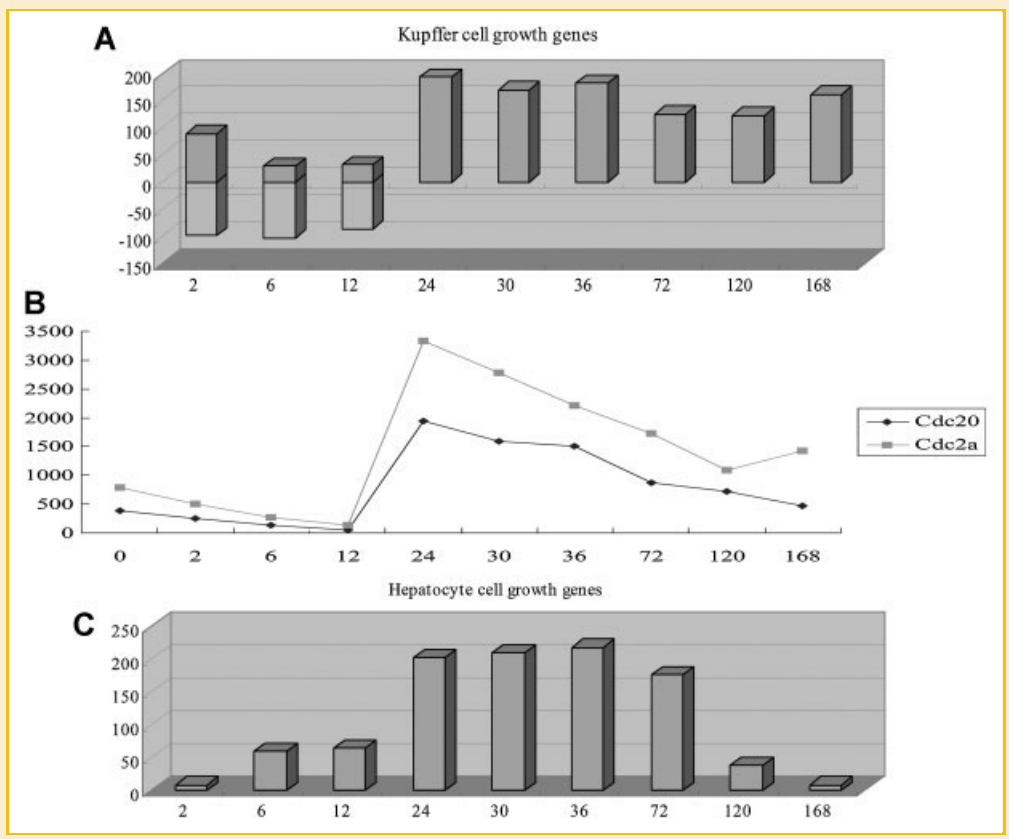

Fig. 4. The temporal functional profile of cell division genes (dark color bars are up-regulated genes, light color bars are down-regulated genes). A: The change of the number of cell division genes in KCs during liver regeneration. B: The expression patterns of cell division genes *cdc2a* and *cdc20*. C: The change of the number of cell division genes in hepatocytes during liver regeneration [Jiang et al., 2011].

KCs needed more time to proliferate. The "cell division" profiles of KCs and hepatocytes are obviously different.

Interestingly, the number of genes in a functional category or temporal functional profile was coincidently similar with the expression pattern of some genes in the category (Fig. 4). *Cdc2a* and *Cdc20* are cell division cycle proteins. Their expression patterns were similar to gene number change pattern. This confirms our previous observation that not only the functions of specific genes but also the number of these genes determine a biological process [Jiang et al., 2011].

Similarly, the temporal functional profile of "signaling" genes displayed the same pattern (Fig. 5). The gene number change was similar to the expression pattern of IL-18, IL-18bp, and related signaling genes, including protein kinase C (*PrKCsb1*, *PrKCsd*), toll-like receptor (*Tlr2*, *Tlr7*, *Myd88*), guanine nucleotide binding protein (*Gnal*, *Gng2*), Ras association domain family (*Rassf4*), purinergic receptor (*P2ry12*, *P2ry14*), mitogen-activated protein kinase (*Map3k8*, *Map4k1*), signal transducer, and activator of transcription (*Stat1*), etc.

The "signaling" patterns of KCs and hepatocytes are quite different. In hepatocytes, signaling genes were up regulated immediately after PH (2–12 h) to initiate liver regeneration. Then the number of genes decreased during cell division period and increased again (36–72 h) to terminate liver regeneration. In KCs, the number of up-regulated signaling increased initially but then these genes were down regulated and the number of down-regulated genes increased until termination phase (120–168 h) when up-regulated genes increased again. For hepatocytes, no signaling genes were down regulated before cell proliferation, implying the up-regulation of signaling initiated hepatocyte proliferation. Whereas for KCs, so many signaling genes were down regulated before cell proliferation, implying the overall role of KCs signaling is inhibiting rather than enhancing cell proliferation. This is consistent with the result of KC depletion experiments.

The temporal functional profile of "immune response" genes clearly showed KCs were partial dedifferentiated during liver regeneration (Fig. 6), just as hepatocytes [Jiang et al., 2011]. The expression patterns of many immune response genes, such as

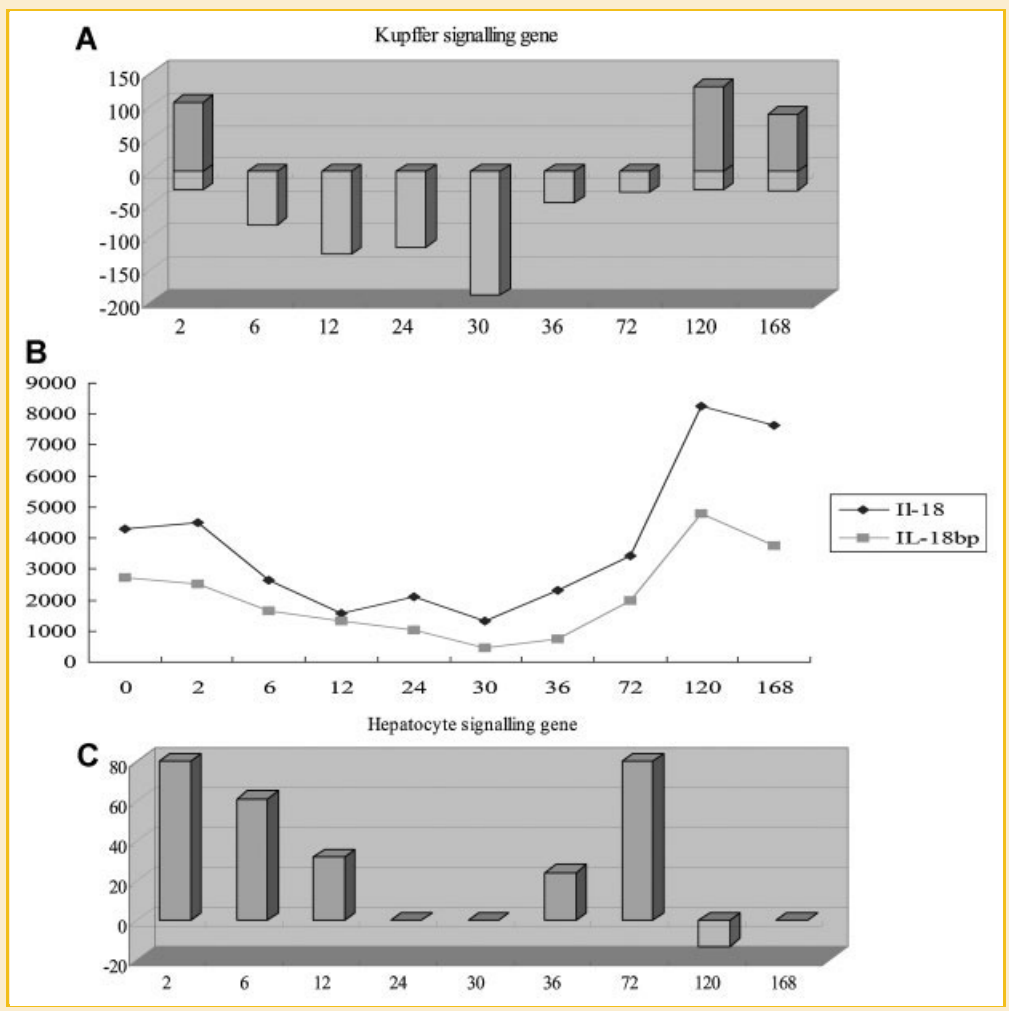

Fig. 5. The temporal functional profile of signaling genes. A: The change of the number of signaling genes in KCs during liver regeneration. B: The expression patterns of signaling genes IL-18 and IL-18bp. C: The change of the number of signaling genes in hepatocytes during liver regeneration [Jiang et al., 2011].

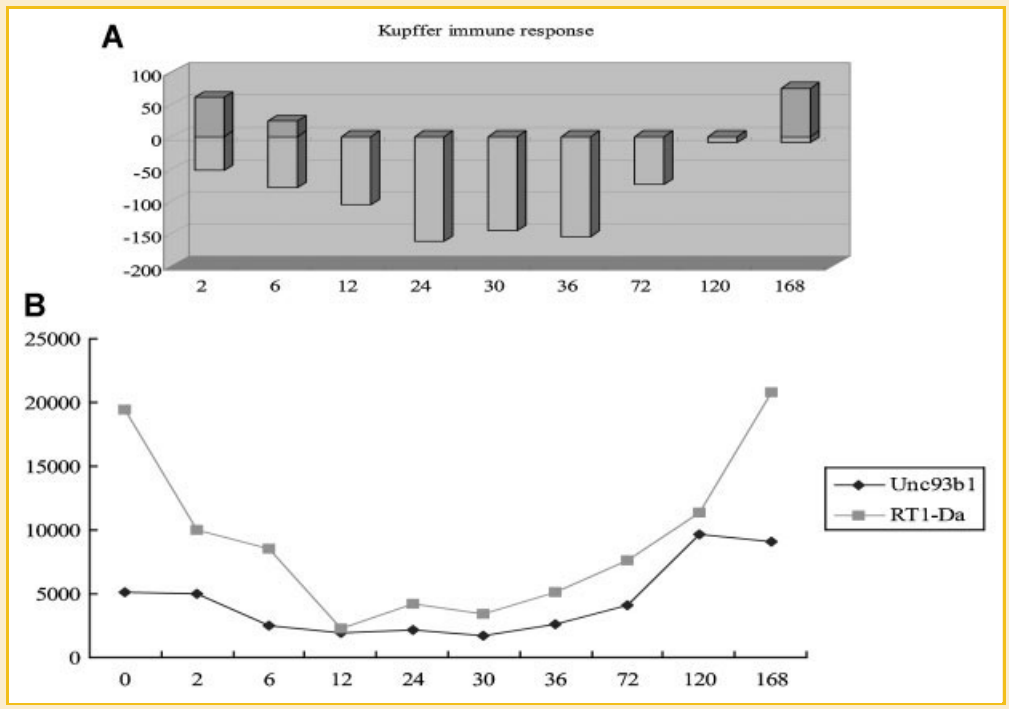

Fig. 6. The temporal functional profile of immune response genes. A: The change of the number of immune response genes in KCs during liver regeneration. B: The expression patterns of immune response genes *Unc93b1* and *RT1-Da*.

Unc93b1, *RT1-Da*, were also similar to that of temporal functional profile.

DISCUSSION

The major difference of the two KC depletion experiments by injection of Cl_2MBP is the time for KC depletion. In Boulton's experiments, rats were administrated Cl_2MBP 24 h before PH [Boulton et al., 1998], while in Takeishi's experiments mice were injected Cl_2MBP 3 days after PH [Takeishi et al., 1999]. We speculated that it was the time that caused the different results.

Rai et al showed KC depletion by gadolinium chloride (GdCl) enhanced liver regeneration after PH in rats and proposed that IL-10 produced by KCs increases TNF mRNA instability and therefore inhibits liver regeneration [Rai et al., 1996, 1997]. KC depletion abolishes induction of IL-10 and permits sustained overexpression of TNF-α. However, as shown in Figure 3A, while IL-10 is only lowly expressed in normal intact liver, it is difficult to explain why KC depletion from normal resting liver without PH results in hepatocyte proliferation in Boulton's experiments.

TGF-β_1 was also used to explain the enhancing effect of KC depletion [Rai et al., 1997; Boulton et al., 1998] because of its inhibitory effect on hepatocyte proliferation. However, TGF-β is expressed in a number of cell types in the liver, predominantly in stellate cells [Michalopoulos, 1997]. The decrease in TGF-β expression after KC depletion was modest [Boulton et al., 1998]. Moreover, the expression of TGF-β in KCs during 1–3 days after PH was not significantly changed compared with normal liver (data not shown). So it is difficult to explain Takeishi's results (1999).

IL-18 is a member of the IL-1 family that was originally described as an inducer of interferon γ (IFN-γ) production from T cells in the presence of IL-12 [Finotto et al., 2004]. IL-18 is produced by KCs, macrophages, B cells, and dendritic cells on lipopolysaccharide (LPS) stimulation [Ghayur et al., 1997]. IL-18 transgenic mice showed reduced relative liver weight and increased hepatocyte apoptosis [Finotto et al., 2004]. IL-18 antisense oligodeoxynucleotide promotes hepatocyte regeneration of partial liver allograft by suppression of IL-18 and IFN-γ production [Xu et al., 2004]. Plasma levels of IL-18 and IL-18bp are elevated in patients with chronic liver disease [Ludwiczek et al., 2002]. Our microarray analysis showed the expression pattern of IL-18 may explain the discrepancy of KC depletion. Because KCs are the major source of IL-18 rather than TNF and IL-6 in the priming phase of liver regeneration [Rai et al., 1996, 1997; Boulton et al., 1998], KC depletion prior to PH enhances liver regeneration. So IL-18 is a potential inhibitor of hepatocyte proliferation and inducer of hepatocyte apoptosis. The role of IL-18 needs to be studied in further experiments.

The down-regulation of signaling genes in KCs before cell proliferation phase, contrary to the up-regulation of signaling genes in hepatocytes [Jiang et al., 2011], also implied the signaling of KCs inhibited the proliferation of themselves. However, in proliferation phase, because of the increased expressions of TGF-α and IL-6, which induced the production of HGF in stellate cells [Kariv et al., 2003], KCs may play a positive role to enhance hepatocyte growth.

It is interesting that the expression patterns of some key genes in KCs were similar to the number of genes in the specific functional category, which was first reported in hepatocytes [Jiang et al., 2011]. It seems that a biological process is regulated not only by the expression change of some specific genes but also by the number of the genes with similar functions involved in the process. Not only the expression of some genes, but also the number of genes involved is important for a process. For those key genes participating in the whole process, the expression patterns may be similar to the temporal functional profile. The mechanisms are still unknown.

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