



Branches of the NF- κ B signaling pathway regulate proliferation of oval cells in rat liver regeneration

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ABSTRACT. The NF- κ B (nuclear factor κ B) pathway is involved in the proliferation of many cell types. To explore the mechanism of the NF- κ B signaling pathway underlying the oval cell proliferation during rat liver regeneration, the Rat Genome 230 2.0 Array was used to detect expression changes of NF- κ B signaling pathway-related genes in oval cells. The results revealed that the expression levels of many genes in the NF- κ B pathway were significantly changed. This included 48 known genes and 16 homologous genes, as well as 370 genes and 85 homologous genes related to cell proliferation. To further understand the biological significance of these changes, an expression profile function was used to analyze the potential biological processes. The results showed that the NF- κ B pathway promoted oval cell proliferation mainly through three signaling branches; the tumor necrosis factor alpha branch (TNF- α pathway), the growth factor branch, and the chemokine branch. An integrated statistics method was

used to define the key genes in the NF- κ B pathway. Seven genes were identified to play vital roles in the NF- κ B pathway. To confirm these results, the protein content, including two key genes (TNF and FGF11) and two non-key genes (CCL2 and TNFRSF12A), were analyzed using two-dimensional gel electrophoresis and MALDI-TOF/TOF mass spectrometry. The results were generally consistent with those of the array data. To conclude, three branches and seven key genes were involved in the NF- κ B signaling pathway that regulates oval cell proliferation during rat liver regeneration.

Key words: Rat liver regeneration; NF- κ B signaling pathway; Key genes; Oval cell proliferation; Gene expression profile

INTRODUCTION

The liver is comprised of liver parenchymal cells and liver non-parenchymal cells. Liver non-parenchymal cells consist of oval cells, Kupffer cells, and other cell types. Oval cells are a kind of stem cells with potential to differentiate into other types of liver cells including hepatocytes (Strain and Crosby, 2000; Yin et al., 2014), biliary epithelial cells, as well as intestinal epithelial cells, pancreatic cells, microglia, and astrocytes (Alison et al., 1996). Normally, the majority of adult liver oval cells are in the quiescent state. After an injury or partial hepatectomy (PH), they can rapidly enter the cell cycle and play a complementary role to the proliferation of hepatocytes (Yamazaki et al., 2011; Gong et al., 2013).

The liver has an outstanding capacity of regeneration. This process involves various physiological and biochemical activities such as cell activation, de-differentiation, proliferation and regulation, re-differentiation, and rebuilding of the structure and function (Fausto et al., 2006). This regeneration is regulated by many signaling pathways (Zhang et al., 2014).

The NF- κ B (nuclear factor κ B) signaling pathway includes three major branches; the tumor necrosis factor alpha branch (TNF- α pathway), the growth factor branch, and the chemokine branch (Bassères and Baldwin, 2006). The branch of TNF- α promotes cell proliferation through TNF- α \rightarrow TNFR1 \rightarrow TRADD \rightarrow TRAF6 \rightarrow IKK \rightarrow IKB \rightarrow NF- κ B (Wang and Xiao, 2007). The growth factor branch promotes cell proliferation through growth factors \rightarrow RTK \rightarrow Ras \rightarrow PI3K \rightarrow Akt \rightarrow NF- κ B (Dong et al., 2007), while the branch of chemotactic factor promotes cell proliferation through Chemokine \rightarrow CR \rightarrow G α I \rightarrow PI3K \rightarrow Akt \rightarrow NF- κ B (Van Sweringen et al., 2011). Generally, the NF- κ B signaling pathways contain 184 genes, and cell proliferation includes 1407 genes. Because many genes are involved in the NF- κ B signaling pathways and cell proliferation with complex gene-interactions, systematic biology methods have been used to analyze the regulatory networks and the sequence of NF- κ B signaling pathways in regulating oval cell proliferation.

The NF- κ B signaling pathway includes three branches, however, little is known about the function of these branches in the regulation of oval cell proliferation during liver regeneration (LR). In this study, we used the Rat Genome 230 2.0 Array to detect the gene expression profile of oval cells isolated from ten time points during rat LR (Guruge et al., 2006). The synergy among the genes involved in NF- κ B signaling pathway and cell proliferation were calculated with a mathematical model of the expression profile function (E_p). The key genes in the NF- κ B signaling pathway and cell proliferation during rat LR were defined by integrated statistics methods and the mechanism of the key genes were analyzed by Pathway studio 8.0 (Nikitin et al., 2003).

MATERIAL AND METHODS

Isolation and identification of oval cells of rat LR

The experimental procedure has been described in previous papers (Lu et al., 2009). Briefly, rats of NO, PH and sham-operated (SO) groups were prepared. The purity of the isolated oval cells was identified with the immunocytochemical analysis of two marker proteins OC2 and OV6 (Lu et al., 2009). The purity of the oval cells was $\geq 95\%$. The whole handling procedure was performed in accordance with the current Animal Protection Law of China.

Rat Genome 230 2.0 array detection and data analysis

Total RNA was extracted and purified following the protocols as previously described (Xu et al., 2010). Briefly, biotin-labeled cRNA was synthesized and then digested into 35-200-bp cRNA fragments using GeneChip *In Vitro* Transcript Labeling Kit (ENZO Biochemical, New York, NY, USA). The prehybridized Rat Genome 230 2.0 Array was prepared following the Affymetrix protocol and hybridized with the cRNA. The hybridized arrays were washed and stained in GeneChip fluidics station 450 (Affymetrix Inc., Santa Clara, CA, USA). The arrays were then scanned and imaged with a GeneChip scanner 3000 (Affymetrix Inc.). Those images showing gene expression abundance were converted into signal values, signal detection values (present, marginal, and absent) and experiment/control values (Ri), using Affymetrix GCOS 2.0. Present (P), marginal (M), and absent (A) values were defined as $P < 0.05$, $P < 0.065$, and $P > 0.065$, respectively. The data of each array were initially normalized by scaling all signals to a target intensity of 200. To minimize the technical error from the array analysis, each sample was tested at least three times by the Rat Genome 230 2.0 array, and the average value was used in the data analysis. The statistical analyses of these data were done using GeneMath (Zhu et al., 2015), GeneSpring (Dalman et al., 2012), Microsoft Excel (Rogosch et al., 2012), and Pathway studio 8.0 (Almon et al., 2003; Nikitin et al., 2003; Mulrane et al., 2008).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted and purified following previously reported protocols (Xu et al., 2010). Briefly, total RNA was reverse-transcribed using random primers and a reverse transcription kit (Promega, Madison, WI, USA). The primers OC2 (NM_194268.2), OV6 (NM_165849.2), CCND1 (NM_171992.4), and DUSP1 (NM_053769.3) were designed with Primer Express v. 2.0 (Canning et al., 1999). First-strand cDNA samples were subjected to qPCR amplification using SYBR[®] Green I on the Rotor-Gene 3000A (Corbett Robotics, Brisbane, Australia). The copy numbers of target genes in each mL of the sample were calculated according to their corresponding standard curves. The qPCR cycling conditions were as follows: 2 min at 95°C, followed by 40 cycles of 15 s at 95°C, 15 s at 60°C, and 30 s at 72°C. Each sample was analyzed in triplicate.

Identification of NF- κ B signaling pathway- and cell proliferation-related genes

The search terms “NF- κ B signaling pathway” and “cell proliferation” were input into NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu), to collect rat, mouse, and human genes associated with NF- κ B signaling pathway or cell proliferation. The list of genes found

during the initial search, was subsequently compared to and complemented with genes in the biological pathway maps found in GENMAPP (www.genmapp.org), KEGG (www.genome.jp/kegg/pathway.html), and QIAGEN (www.qiagen.com/geneglobe/pathways.aspx) (Salomonis et al., 2007; Antonov et al., 2010). Moreover, NF- κ B signaling pathway-related genes, such as *NFKB1*, *NFKB2*, *RelA*, *RelB*, and *c-Rel*, were input into TRED (<http://rulai.cshl.edu/cgi-bin/TRED/tred.cgi?process=searchTFGeneForm>), LymphTF-DB (<http://www.iupui.edu/ffinterx/activity.php>), and NF- κ B (www.bu.edu) (Childress et al., 2007; Jiang et al., 2007). This was done to find their downstream target genes in rat, mouse, and human, among which cell proliferation-related genes were identified with the NCBI database, and reconfirmed with published articles. BLAST was used to find the homologues of LR-related genes (Sankian et al., 2005), and the unknown genes homologous to NF- κ B signaling pathway and/or cell proliferation-related genes were considered to serve the same function.

Identification of significantly changed genes and LR-related genes during rat LR

The normalized log ratio values of PH were used to calculate the relative value of each gene (ratio values). A gene was considered to have changed significantly when its ratio value was ≥ 3 or ≤ 0.33 , while a non-significant change was considered at ratios between 2.99 and 0.34 during LR (Vardhanabhuti et al., 2006). Furthermore, to analyze the differences in gene expression between PH and SO groups, *t*-tests were used (de Menezes et al., 2004). The genes that were found to be significantly different between PH and SO at least once during LR with a significant ($0.01 \leq P < 0.05$) or highly significant ($P \leq 0.01$) level, were considered LR-related genes.

Analysis of gene synergy

A mathematical model (E_p) established by Xu et al. (2010), was used to analyze the synergy among NF- κ B signaling pathway- and cell proliferation-related genes. In brief, Rat Genome 230 2.0 could detect the expression changes in 23,917 genes of rat oval cells (Wang and Xu, 2010). The gene signal value in PH compared to that in SO groups was considered the ratio value for each gene. The log ratio values for the NF- κ B signaling pathway-related and oval cell proliferation-related genes (as identified above) were calculated. Finally, the log ratio values for these genes were added to the E_p formula to calculate gene synergy.

Exploring and screening of key genes during rat LR

The key genes in rat liver regeneration were determined according to the integrated statistics methods established by Xu et al. (2013). In brief, the 23,917 genes for which expression changes could be detected, together with the ratio value of each gene, was added to twelve formulas (Bhattacharyya Distance, Euclidean distance, Fisher test, Signal to Noise Ratio, Feature Score Criterion, *t*-test, χ^2 test, BSS/WSS, correlation coefficient, Pearson Correlation Coefficient, linear correlation coefficient, and Cosine similarity) to calculate gene expression differences in oval cells of rat LR. The top 1000 genes with maximal expression difference were integrated in each formula. The top 1000 genes with the highest occurrence frequency in all algorithms were obtained. Only the genes that were found among the identified 1000 genes that were also found to be involved in NF- κ B signaling pathway and oval cell proliferation were considered key oval cells genes in rat LR.

Analysis of interactions among key and other genes

Pathway studio 8.0 (Nikitin et al., 2003) was used to analyze and establish the interaction network firstly for the whole genome genes and, subsequently, for the identified key genes of rat oval cells. The key genes were then added to the interaction network of the whole genome genes to identify their location, nodes, relationships, and effects. Finally, the information derived from the above mentioned steps was used to enrich and rebuild the interaction network of the NF- κ B signal pathway- and oval cell proliferation-related genes, and then to identify the functions and pathways of the key genes.

Verification of the proteins expressed by key genes using mass spectrometry

To validate the reliability of the array analysis results, several key proteins were selected for an additional assay. The total proteins from the oval cells of regenerating livers were separated and displayed using two-dimension gel electrophoresis. The content and expression of these proteins, such as TNF- α and its receptor TNFRSF12A, chemokine CCL2, and fibroblast growth factor FGF11, were analyzed by MALDI-TOF/TOF.

RESULTS

Expression change of NF- κ B signaling pathway genes related to oval cell proliferation

Based on NCBI, RGD, and the biological pathway maps in KEGG and QIAGEN, 184 genes were identified to be involved in NF- κ B signaling pathway. The Rat Genome 230 2.0 Array used in this study contained 166 of these genes and 40 unknown homologous genes. The comparison of the PH and SO groups using *t*-tests indicated that 64 of the 166 genes were had a significantly different oval cell LR and were defined as liver regeneration-related genes. Of these 64 genes, 34 known and 12 unknown homologous genes were up-regulated; 12 known and 3 unknown homologous genes were down-regulated; and 2 known genes and 1 unknown homologous genes were up/down-regulated ([Table S1](#)).

In addition, according to the public databases mentioned above, 1407 genes are involved in cell proliferation. Among these, 1407 known and 272 unknown homologous genes were included in the Rat Genome 230 2.0 Array. The comparative analysis of the PH and SO groups indicated that 455 genes showed significant changes in the LR oval cell and were identified as liver regeneration-related genes. Among these, 260 genes, including *Abcb1* and 49 unknown homologous genes, were up-regulated, and 97 genes, such as *Acvr1c* and 34 unknown homologous genes, were down-regulated, while 13 genes, including *Bub1* and 2 unknown homologous genes, were up/down-regulated.

The data from TRED and LymphTF-DB showed that 903 genes are regulated by the NF- κ B signaling pathway. Of these, 469 are contained in Rat Genome 230 2.0 Array, and 192 known and 32 unknown homologous genes were cell proliferation-related target genes. The analysis of differential gene expression in the PH and SO groups indicated that 84 genes were significantly different in the LR oval cell and were considered LR-related genes. Among these, 49 known genes, including *Areg* and eight unknown homologous genes, were up-regulated and 19 known genes such as *Ccl5* and 5 unknown homologous genes, *Il6*, *Nos2*, *Hmox1*, *Pthlh*, and *Tnf*, were down-regulated (Table 1).

Table 1. Expression changes in oval cell proliferation-related genes regulated by the NF- κ B signaling pathway during rat liver regeneration.

Gene symbol	Recovery time (h) after partial hepatectomy								
	2	6	12	24	30	36	72	120	168
Tnf	0.13	4.96	1.02	0.61	1.21	1.46	0.43	1.45	0.56
Tnfrsf12a	10.86	5.65	7.45	1.12	4.51	5.19	1.22	3.37	0.78
Ikbkb	4.61	2.54	2.38	3.74	3.77	2.07	3.11	2.85	2.19
Nfkbib	1.25	1.11	1.02	0.92	0.66	1.06	1.19	0.93	0.69
AA957129	3.24	2.80	1.21	2.09	1.71	1.55	1.91	0.64	1.89
Rela	1.19	1.33	1.07	1.06	0.85	0.97	0.96	1.02	0.88
BF419700	3.42	2.61	3.56	3.99	0.80	3.25	5.64	3.69	3.01
Ctgf	3.16	5.95	1.50	2.43	0.66	4.86	1.46	1.67	3.80
Egf	0.69	0.36	0.20	0.72	0.89	0.39	1.04	0.41	0.50
Fgf1	0.51	0.23	0.24	0.81	1.67	1.59	0.92	1.33	0.68
Fgf10	0.50	1.14	0.50	0.81	0.85	0.42	0.57	0.82	0.86
AI137604	1.28	0.63	0.40	0.55	1.89	1.17	4.46	0.55	2.73
Fgf11	1.02	1.31	1.08	3.02	2.24	0.75	2.20	1.36	1.45
Fgf12	2.53	0.75	1.40	2.11	1.10	1.86	1.98	1.44	3.01
Fgf13	3.10	2.42	3.14	2.72	5.89	2.78	6.14	5.02	7.83
Fgf17	3.22	2.21	3.11	1.47	0.82	0.85	1.35	1.49	1.77
Fgf18	0.76	1.08	1.30	0.79	1.17	5.49	1.53	1.09	0.76
Fgf21	0.78	3.39	2.37	1.13	2.70	0.89	0.63	1.18	0.95
Fgf4	2.63	2.07	1.82	1.43	2.14	2.91	3.15	3.38	3.25
Fgf7	1.08	2.79	0.77	0.53	1.49	0.91	2.17	2.05	1.56
AI177304	2.09	4.06	1.23	0.97	1.34	0.98	0.98	3.41	1.81
Fgf8	0.48	0.55	0.38	0.34	0.45	0.12	0.11	0.54	0.14
Pdgfd	1.13	1.11	1.09	5.48	4.91	1.52	1.88	1.67	5.91
Pgf	4.32	5.56	2.87	2.44	4.57	5.29	3.70	5.18	2.86
Tgfb3	18.68	2.94	1.81	1.32	0.73	2.13	2.45	1.50	2.75
Ddr1	1.53	2.18	1.43	1.85	0.98	2.46	7.79	2.11	1.57
Ddr2	3.10	2.28	5.20	2.59	1.87	7.02	3.93	4.55	3.12
Epha4	2.01	3.86	3.13	1.93	3.65	1.56	2.41	2.85	1.95
Epha5	1.72	1.56	1.44	1.15	1.11	1.57	1.51	3.68	1.12
Ephb3	3.98	6.19	0.83	0.94	0.76	1.67	1.14	1.17	1.24
ErbB2	4.18	1.60	1.43	1.16	1.15	1.08	1.37	1.40	1.10
ErbB4	0.20	0.36	0.16	0.13	0.75	0.19	0.22	0.17	0.10
AI454917	0.90	0.49	0.72	0.24	0.40	0.43	1.14	0.11	0.20
Flt1	1.61	1.55	0.87	1.24	1.22	0.67	0.96	1.18	1.42
BI296644	1.11	1.32	0.70	1.52	0.25	0.64	0.64	1.45	2.39
BF415786	3.87	2.50	1.07	1.62	0.38	0.79	0.75	1.18	2.42
BE095854	2.84	0.91	2.85	1.69	0.63	4.41	0.84	4.73	1.50
BI293027	3.87	3.13	0.91	1.57	0.39	0.67	0.68	1.34	2.17
Flt3	1.31	0.66	0.58	1.07	0.27	0.97	0.72	0.93	1.42
Igf1r	0.92	0.80	0.93	0.85	1.30	0.75	0.99	0.78	0.82
BG378641	7.36	5.35	3.86	2.08	1.98	3.41	5.50	4.00	4.26
AW529468	0.80	0.64	1.00	0.94	0.70	0.25	1.15	0.88	0.58

Continued on next page

Table 1. Continued.

Gene symbol	Recovery time (h) after partial hepatectomy								
Kdr	0.60	0.66	0.31	1.22	0.36	0.41	0.51	0.72	2.46
Kit	3.23	12.36	3.18	11.37	0.79	1.45	3.48	3.98	13.63
Lifr	0.42	3.07	0.33	1.42	0.66	0.60	0.60	1.11	1.20
BF563716	0.71	4.72	0.23	3.58	1.44	0.59	0.57	1.42	3.74
AW532142	1.87	10.65	1.06	4.63	2.82	0.94	1.00	2.83	3.70
Ntrk3	1.58	2.01	1.98	0.85	2.42	1.66	2.22	1.85	2.05
BF398408	1.39	1.13	3.11	2.15	1.51	0.50	3.70	0.79	3.04
Pdgfra	0.80	1.34	0.71	3.89	0.85	2.17	0.98	1.73	3.73
Pdgfrb	1.82	1.26	1.22	4.02	1.27	3.07	0.78	1.90	2.49
Ptk7	1.02	1.08	0.91	1.50	0.79	4.00	1.66	2.68	1.30
Tek	0.82	0.84	0.59	1.58	0.54	0.66	0.61	1.18	3.11
Pik3c2g	1.06	0.47	0.29	0.86	0.53	0.39	0.85	0.26	0.61
Pik3c2b	1.18	3.49	1.40	1.81	1.35	2.62	1.46	1.72	1.91
Akt3	2.48	2.31	2.85	3.05	2.42	2.42	2.63	3.11	1.46
AI406827	2.67	3.67	1.83	4.70	0.97	2.37	3.16	2.03	4.36
Cxcl2	1.57	3.74	1.81	1.20	1.20	2.70	0.61	1.92	1.36
Ccl28	2.45	2.34	2.27	1.01	1.31	1.17	3.25	0.96	1.60
Xcl1	0.79	0.33	0.63	0.38	0.28	0.58	0.81	1.07	1.09
Ccl2	1.56	2.61	2.64	0.99	0.97	9.45	1.48	7.28	0.89
Cxcl12	0.31	0.31	0.12	1.24	1.17	0.51	0.80	0.60	0.97
Ccl7	2.69	7.24	3.59	1.43	2.71	21.87	1.69	14.47	1.22
Ccl11	4.39	5.26	0.99	3.26	2.70	11.19	0.96	5.26	1.13
Ccl20	0.66	2.63	4.48	4.57	2.30	17.86	3.24	16.00	0.79
Cxcl14	2.43	2.09	0.84	2.96	3.43	1.75	1.52	3.06	3.67
Cxcl5	3.56	3.16	8.04	0.56	6.10	23.67	4.98	12.93	3.27
Cxcl1	7.30	8.52	5.01	3.55	3.04	7.08	4.23	5.49	1.92
Ccl5	2.07	0.51	1.33	0.72	0.31	0.55	0.83	0.71	1.94
Ccr5	1.63	2.51	4.04	1.19	1.13	1.52	1.30	1.11	2.90
Gnai1	1.48	1.29	4.67	2.26	1.12	2.73	1.49	1.59	3.73
BF289002	1.64	1.14	3.92	2.11	1.14	2.44	1.20	1.44	3.31
Rras2	1.07	1.52	1.74	1.26	1.80	1.29	1.18	1.45	1.46
AI548708	3.52	2.43	6.02	3.06	0.72	1.93	3.07	0.92	2.75

*The values in red represent the expression abundance of up-regulated genes, those in green that of the down-regulated and those in black that of the insignificantly changed. The symbols in yellow ground indicate the unknown genes homologous to the above known genes.

The expression trends of four target genes, including *Oc2*, *Ov6*, *Ccnd1*, and *Dusp1*, detected by qRT-PCR, were compared to the results by gene chip detection. This showed that the expression trends detected by these two methods were generally consistent. Other genes previously detected by RT-PCR in previous studies also gave the same results (Wang and Xu, 2010; Xu and Zhang, 2009), indicating that the array results were reliable for further analysis.

Role of the three branches of NF- κ B signaling pathway in the regulation of oval cell proliferation

The mathematical model E_p was used to analyze the synergy among NF- κ B signaling

pathway-related genes in oval cells. The results demonstrated that the signal transduction activity of the NF- κ B signaling pathway increased during LR, and peaked at 72 h after PH. E_p values of genes related to the TNF- α , growth factor, and chemotactic factor branches of the NF- κ B signaling pathway were also higher in the PH group than in normal control and SO groups. Each peaked at 36, 72, and 36 h after PH (Figure 1).

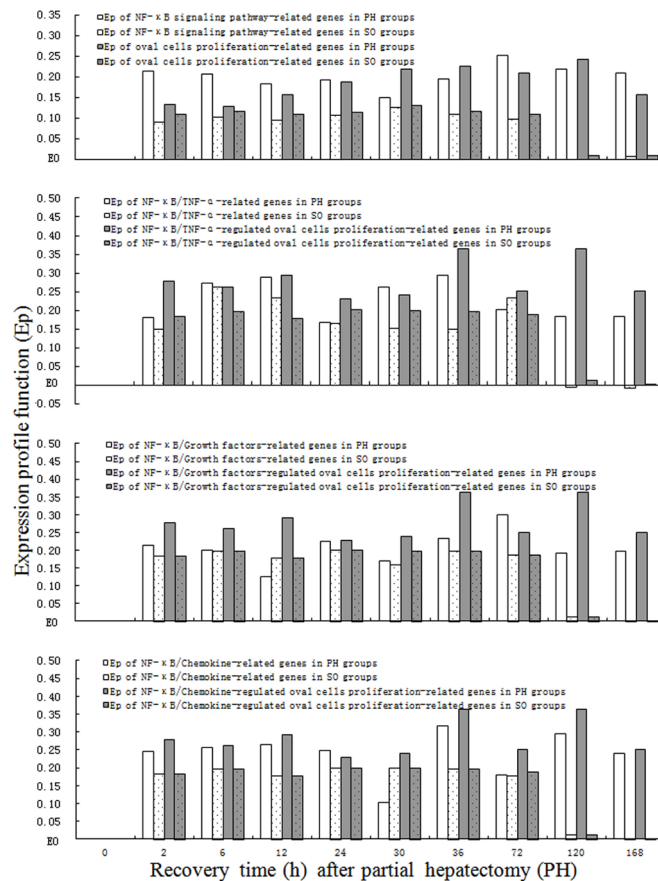


Figure 1. Relationships between the roles of different NF- κ B signaling pathway branches and that of their regulated oval cell proliferation.

E_p values of oval cell proliferation-related genes in the PH group were greater than those in the SO group, and peaked at 30 h after PH. Furthermore, E_p values of genes related to the TNF- α , growth factor, and chemotactic factor branch-regulated oval cell proliferation were also higher in the PH group than in the SO group, and were all found to be significantly higher at 36 h than at all other time points (Figure 1).

The E_p model was also used to analyze the relationships between the signal transduction activity of the NF- κ B signaling pathway and cell proliferation. E_p values of the NF- κ B signaling pathway-related oval cell proliferation-related genes were higher in the PH group than in the SO group. In particular, the E_p values of the regulatory oval cell proliferation-related genes associated

with the TNF-α, growth factor, and chemotactic factor branches were higher in the PH group than in the SO group (Figure 1).

Key genes in the NF-κB signaling pathway and oval cell proliferation, and their functions during rat LR

Several methods of integrated statistics were applied to analyze the difference in gene expression among the PH and SO groups. The results indicated that maximum differences were found in seven genes; *Tnfrsf12a*, *Egf*, *Xcl1*, *Ccl2*, *Cxcr4*, *Ccnd1*, and *Dusp1*, demonstrating that these genes may play important roles in rat LR. TNF-α and its receptor *Tnfrsf12a*, both up-regulated, together activate the NF-κB/TNF-α branch. Chemotactic *Xcl1* was down-regulated, whereas chemotactic *Ccl2* was up-regulated. Through their interaction with chemotactic receptor *Cxcr4*, chemotactic *Xcl1* and *Ccl2* may promote the expression of the cell proliferation-stimulatory genes *CCND1*, and restrain the expression of the cell proliferation-inhibitory gene, *DUSP1* (Figure 2).

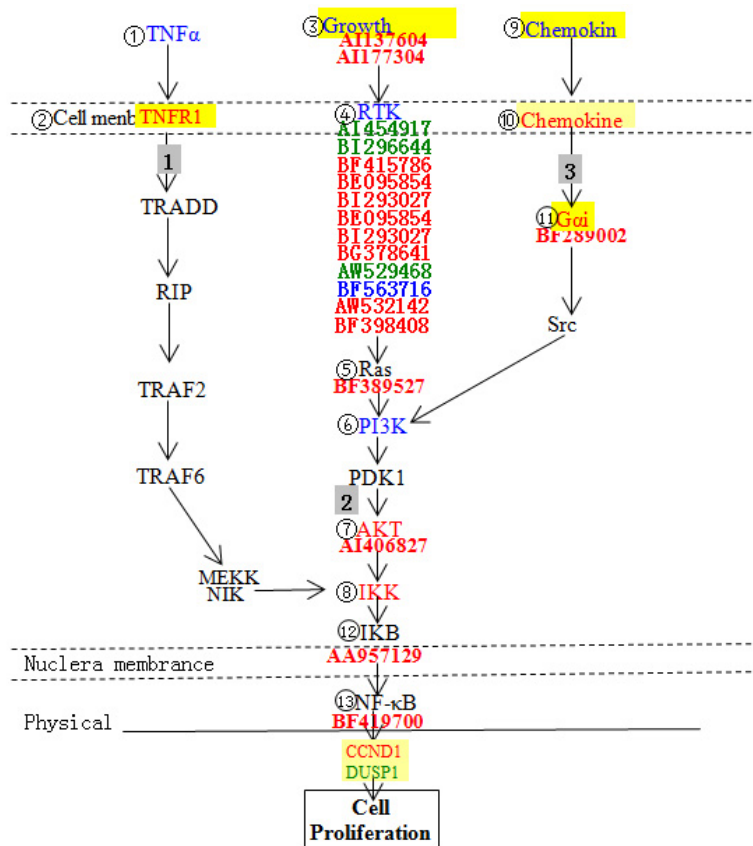


Figure 2. Regulatory effect of the three branches of the NF-κB signaling pathway on oval cell proliferation. The symbols in red represent significantly up-regulated genes, green means down-regulated, blue denotes up/down-regulated and black is insignificantly changed. Highlights in red bold indicate that the gene is homologous to the one above. Highlights in black bold show the cell proliferation activity studied in this paper. The symbols highlighted in yellow are the identified key genes. Circled numbers correspond to the ordinal protein numbers in [Table S1](#).

Proteins expressed by the key genes during rat LR

The reliability of the Array results was qualitatively and quantitatively tested on several proteins using two-dimensional gel electrophoresis and MALDI-TOF/TOF. We found that TNF- α was up/down-regulated, and its receptor TNFRSF12A, chemokine CCL2, and fibroblast growth factor FGF11 were all up-regulated in the oval cells of rat LR. These results were consistent with the results which were predicted by Array. (Xu CS, unpublished results).

DISCUSSION

In this study, the expression profiles of NF- κ B signal pathway-related genes was detected using Rat Genome 230 2.0 Array at transcriptional level during rat LR. Several systems biology methods were combined to analyze the potential physiological functions of these genes. The results revealed that three branches of the NF- κ B signal pathway promote cell proliferation.

The TNF- α branch and its regulatory oval cell proliferation activity were enhanced during rat LR (Figure 1). The key gene, *Tnfrsf12a*, was up-regulated at 2-12, 30-36, and 120 h, *Ikbkb* (inhibitor of kappa light polypeptide gene enhancer in B-cells) was up-regulated at 2, 24-30, and 72 h and the mRNA level of *BF419700* (the homologue of transcription factor NF- κ B family member *RELA*) increased at 2, 12-24, and 36-168 h (Table S1). The results above were consistent with those found by Yuceturk et al. (2007). Based on the above results, a possible relationship between the TNF- α branch and oval cell proliferation was proposed as follows; the over-expression of TNF- α and its receptor, TNFRSF12A, activates *Ikbkb*, leading to the activation of *AA998997* (the homologous gene of the transcription factor NF- κ B family member *RELA*). This, in turn, stimulates the activity of oval cell proliferation by up-regulating the expression of the cell proliferation-promoting gene *CCND1* and down-regulating the expression of the cell proliferation-inhibiting gene *DUSP1*. As a result, oval cell proliferation is significantly enhanced during rat LR.

The expression profiles of the growth factor branch-related genes showed that the fibroblast growth factor Fgf10's homologous genes *A1137604* and *A1177304* were both up-regulated at 72 h. Similarly, *Ras*' homologous gene *A1548708* was up-regulated at 2, 12-24, and 72 h, and *Akt3* (v-akt murine thymoma viral oncogene homolog 3) and its homologous gene *A1406827* were up-regulated at 24 and 72 h; 6, 24, 72, and 168 h (Table S1). These results were consistent with those found in previous studies (Kato et al., 2007). The physiological processes were assessed using methods of systematic biology. The results showed that both the growth factor branch and its regulatory oval cell proliferation activity were elevated during rat LR (Figure 1). The possible connection between this branch and oval cell proliferation activity may act according to the following: growth factors' homologous genes and their receptor genes may promote the overexpression of *Ras*' homologous gene *A1548708*. This gene, in turn, activates *Akt3* and its homologous gene *A1406827*, leading to an enhanced oval cell proliferation through up-regulation of the cell proliferation-promoting gene *CCND1*, and down-regulation of the cell proliferation-inhibiting gene *DUSP1*.

Array analysis of the chemotactic factor branch-related genes illustrated that the key gene *Ccl2* was up-regulated at 36 and 120 h, guanine nucleotide binding protein (G protein) and *Gnai1* (alpha inhibiting 1) and its homologous gene *BF289002* were both up-regulated at 12 and 168 h during rat LR (Table S1). These results were consistent with the study of Van Sweringen et al. (2011). Both the chemotactic factor branch and its regulatory oval cell proliferation activity were enhanced in rat LR (Figure 1). The results suggest that an up-regulation of the key gene *Ccl2* leads to the activation of *Gnai1*, which in turn activates *Akt3*. Activation of *Akt3* results in up-regulation of

BF419700 (homologue to transcription factor NF- κ B family member *RELA*). The latter augments oval cell proliferation activity through up-regulating the expression of the cell proliferation-promoting gene *CCND1*, and down-regulating the expression of cell proliferation-inhibiting gene *DUSP1*, ultimately resulting in enhanced oval cell proliferation during rat LR.

This study demonstrated that three branches and seven key genes in the NF- κ B signaling pathway regulate the proliferation of oval cells during rat LR. In the future, gene knockout, overexpression, RNA interference, and similar methods will be used to further study the detailed mechanisms with which each of these NF- κ B signaling pathway branches regulate oval cell proliferation in rat LR.

Conflicts of interest

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. Expression changes in NF- κ B signaling pathway-related genes during rat liver regeneration.

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