Portal vein ligation alters coding and non-coding gene expression in rat livers

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Complete List of Authors:	Li, Bin; Second Military Medical University, Eastern Hepatobiliary Surgery Hospital Zhu, Yan; Second Military Medical University, Changhai hospital Xie, Lei; Second Military Medical University, Eastern Hepatobiliary Surgery Hospital Hu, Shuyang; Second Military Medical University, Eastern Hepatobiliary Surgery Hospital Liu, Shupeng; Second Military Medical University, Changhai hospital Jiang, Xiaoqing; Second Military Medical University, Eastern Hepatobiliary Surgery Hospital
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Portal vein ligation alters coding and non-coding gene expression in rat livers**Running title: PVL alters gene expression in rat livers**

Bin Li¹, Yan Zhu², Lei Xie¹, Shuyang Hu¹, Shupeng Liu³, Xiaoqing Jiang¹

1, Biliary Tract Surgery Department I, Eastern Hepatobiliary Surgery Hospital, Secondary Military Medical University, Shanghai, China

2, Department of Pathology, Changhai Hospital, Secondary Military Medical University, Shanghai, China

3, Clinical Research Center, Changhai Hospital, Secondary Military Medical University, Shanghai, China

Corresponding authors: Xiaoqing Jiang, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, 225 Changhai Road, Shanghai, China; Phone: 86-21-81875281; E-mail: jxqehbh@sina.com; or Shupeng Liu, Changhai Hospital, Second Military Medical University, 168 Changhai Road, Shanghai 200433, China; Phone: 86-21-31162099; E-mail: lshu_p@aliyun.com.

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Bin Li and Yan Zhu contributed equally to this article.

Abstract

Portal vein occlusion increases the resectability of initially unresectable liver cancer by inducing hypertrophy in non-occluded liver lobes. However, the mechanisms of how portal vein occlusion induces hepatic hypertrophy remain unclear. A cDNA microarray was used to identify the gene expression signatures of ligated (LLs) and non-ligated lobes (NLLs) at different time points after portal vein ligation (PVL). The results of a bioinformatics analysis revealed that LLs and NLLs displayed different gene expression profiles. Moreover, the expression levels of both coding and non-coding RNA were different between LLs and NLLs at different time points after PVL. A Series Test of Cluster analysis revealed that the No. 22 and No. 5 expression patterns, which showed altered expression at 24 h and maintained this altered expression over the following 14 d, had the lowest *P* value and the largest number of differentially expressed genes in both LLs and NLLs. The results of a GO analysis showed the activation of hypoxia pathways in LLs and the activation of cell proliferation and cell cycle pathways in NLLs, suggesting the involvement of these pathways in PVL-induced hepatic hypertrophy and regeneration. These results provide insight into the molecular mechanisms underlying hepatic hypertrophy and regeneration induced by portal vein occlusion, and they identify potential targeting pathways that can promote the clinical application of PVL in liver cancer therapy.

Keywords: portal vein ligation, microarray, rat model, liver regeneration

Introduction

Complete hepatic tumor resection is the main option for curative treatment of liver malignancies and provides patients with a chance for long-term survival (Utsunomiya et al. 2014). Up to 70% of patients are unsuitable for resection because of insufficient remnant liver volume, which always leads to liver failure and increased postoperative morbidity after major liver resection (She and Chok 2015). Portal vein occlusion via embolization (PVE) or ligation (PVL) induces hypertrophy in non-occluded liver lobes and increases the resectability of an inadequate functional remnant liver volume (Siriwardana et al. 2012). It has been reported that PVE is associated with a minimal mortality rate in patients receiving extended hepatectomy (Shindoh et al. 2014). However, the mechanisms of how PVE/PVL induces contralateral hepatic hypertrophy are still poorly understood.

In patients, PVE has been reported to induce apoptosis in the embolized lobe and proliferation in the non-embolized lobe, which may involve the altered expression of transforming growth factor- α (TGF- α) and transforming growth factor- β (TGF- β) in livers after PVE (Kusaka et al. 2006). Changes in portal flow and cytokine expression after PVE/PVL were also reported in animal models. A reduction in left liver flow and an increase in right liver flow were observed when left portal vein stenosis was performed—results that were further verified by the dilation of the portal branches in the non-embolized lobe (Kawai et al. 2002; Rocheleau et al. 1999). Cytokines and transforming growth

factors, such as TGF- α and IL6, in both serum and remnant livers were all elevated after PVE/PVL (Garcia-Perez et al. 2015; Kusaka et al. 2004). Hepatic regeneration was promoted by perioperative treatment with HGF, suggesting that the altered genes were involved in liver atrophy and hypertrophy after PVE/PVL (Mangieri et al. 2016). Moreover, the expression of activin beta family members (A, C and E) was elevated in the early stage of PVL, indicating the involvement of activin signaling in liver regeneration after PVL (Tashiro 2009). Another study reported that mitochondrial DNA replication and transcription were both enhanced in the non-ligated lobe, which increased the energy supply for liver regeneration (Shimizu et al. 1995). These findings suggest that a complex signal transduction network is activated in livers after PVE/PVL to induce contralateral hepatic hypertrophy and to prevent post-hepatectomy liver failure (Yokoyama et al. 2007). Although many factors have been reported to be involved in PVE/PVL-induced hepatic hypertrophy and liver regeneration, a systematic investigation of the gene expression profiles in livers after PVE/PVL using high-throughput platforms, such as cDNA microarrays, is lacking.

In the present study, we established a rat model of PVL and identified the gene expression signatures of ligated and non-ligated lobes at different times after PVL using cDNA microarrays. We found that PVL induced atrophy in ligated liver lobes (LLs) and hypertrophy in non-ligated liver lobes (NLLs) in rats. At different times after PVL (24 h, 7 d and 14 d), both LLs and NLLs

displayed unique gene (coding and non-coding) expression profiles assessed by cluster analysis based on cDNA microarray data. In the first 24 h after PVL, the expression of most genes was altered. The results of a subsequent GO analysis of these differentially expressed genes showed enrichment of hypoxia, cell proliferation and apoptosis pathways. The present study identified genes involved in PVL and proposes the pathways through which PVL induces hepatic hypertrophy and regeneration. These results may provide further insight into the hepatic hypertrophy and regeneration induced by PVL at the molecular level and potential targeting pathways to promote the clinical application of PVL in liver cancer therapy.

Materials and methods

Animals

Male Sprague–Dawley rats, 6–8 weeks old and 140~220 g in weight, were obtained from the Experimental Animal Center of the Second Military Medical University. All rats were maintained in a pathogen-free facility with alternate light and dark conditions and were given standard laboratory rodent chow. All animal experiments (SCXK(HU)2013-0016) were performed in accordance with the institutional guidelines for animal care.

PVL model

The PVL model was established as previously reported (Dhar et al. 2015). Briefly, abdominal anesthesia was administered using 10% chloral hydrate at a 0.035 ml/kg body weight dose. The abdomen was opened via a midline

incision, and blood vessels including the portal branches and arteries were identified with the help of an operating microscope. The portal vein of the left lobe, the middle lobe and the hepatic papillary was ligated with a double surgical suture to block the blood supply. The PVL model was complete when the color of the right lobe remained light brown, and the other lobes turned dark brown. At 24 h, 3 d, 7 d and 14 d after PVL, the animals were sacrificed by cervical dislocation. Whole livers were dissected, the total liver weight was measured, and then the individual lobes were weighed separately. Liver tissues were kept in liquid nitrogen for future use.

Microarray analysis

LLs and NLLs were obtained from rats at different time points (24 h, 7 d and 14 d) after PVL. Normal liver tissues were used as the controls. RNA was isolated from these tissues using TRIzol® Reagent (Invitrogen Life Technologies, Carlsbad, CA) and sent to KANGCHEN Corporation for microarray analysis using the Rat 4 x 44K LncRNA expression array. Data were extracted using Agilent Feature Extraction software. Normalization and further data analysis were performed using Agilent GeneSpring GX v11.5.1 software.

Identification of differentially expressed genes

The random-variance model (RVM) F-test was applied to identify differentially expressed genes between the normal tissues and LLs or NLLs from different time points (24 h, 7 d and 14 d). After the significance analysis

and FDR analysis, we selected the differentially expressed genes according to their P value threshold ($P < 0.05$) (Clarke et al. 2008).

Series test of cluster

A Series Test of Cluster (STC) was used to analyze the dynamic expression of genes in LLLs and NLLs at different time points, as previously reported (Su et al. 2013).

Gene ontology

To analyze the differentially expressed genes at the functional level, GO enrichment analyses were performed using the DAVID online tool. $P < 0.05$ was considered statistically significant (Liang et al. 2016).

Statistical analyses

Statistical analyses were performed with SPSS 13.0 for Windows (SPSS). Quantitative variables were analyzed using a t -test. $P < 0.05$ was considered statistically significant.

Results

PVL induced atrophy in LLLs and hypertrophy in NLLs in rats

The rat PVL model was established by ligating the portal vein of the left lobe, the middle lobe and hepatic papillary. All the rats survived the procedure and recovered during the postoperative period. At different time points (24 h, 3 d, 7 d and 24 d) after the ligation of the portal vein, liver tissues from sacrificed rats ($n=6$) were weighed. The results showed that the highest whole liver weight (WLW)/body weight (BW) ratio occurred at 7 d after PVL; no significant

difference was found at 24 h or 3 d (Figure 1A). The NLLL weight (NLLLW)/BW ratio increased along with the time after PVL, whereas the LLL weight (LLLW)/BW ratio decreased (Figure 1B). Similar results were obtained when the NLLLW/WLW and LLLW/WLW ratios were analyzed (Figure 1C). These results indicated that PVL induced hypertrophy in NLLLS and atrophy in LLLs in rats.

PVL induced gene expression changes in rat livers

To investigate gene expression in NLLLS and LLLs at different time points after PVL (0 h, 24 h, 7 d and 14 d), tissues were obtained and sent for cDNA microarray analysis. Multiple genes were found to be differentially expressed in LLLs compared with the corresponding NLLLS at all three time points (Figure 2A and B). According to the criteria of a fold change ≥ 2.0 and $P < 0.05$, there were 1296, 500 and 530 differentially expressed genes between LLLs and NLLLS at 24 h, 7 d and 14 d, respectively (Figure 2C). Additionally, long non-coding RNAs were analyzed, which revealed that 371, 276 and 165 non-coding RNAs were differentially expressed between LLLs and NLLLS at 24 h, 7 d and 14 d, respectively (Figure 2D). These results indicated that PVL changed the expression of coding and non-coding genes in both ligated and non-ligated liver lobes.

Gene expression changed over time after PVL

To investigate gene expression at different times after PVL, we compared changes in the genes expressed in LLLs or NLLLS with those in normal liver. A

hierarchical clustering analysis showed that both the LLLs and NLLs had unique mRNA (Figure 3A and B) and non-coding RNA expression profiles at each time point (Figure 3C and D). Additionally, an STC analysis was applied to analyze the dynamic gene expression pattern in relation to the time after PVL. At four different time points, a total of 26 possible dynamic expression profiles were identified. For mRNA, 11 of 26 expression profiles were statistically significant in the LLLs, and 13 profiles were statistically significant in the NLLs (Figure 4A and B). Among these profiles, 9 profiles were significantly changed in both LLLs and NLLs. Profiles No. 22 and No. 5 had the lowest *P* values and the largest number of differentially expressed genes in both groups (Table 1). The expression of genes contained in these two profiles was altered at 24 h, and these expression levels were maintained for the following two weeks. For long non-coding RNAs, 9 profiles in LLLs and 10 profiles in NLLs were significantly changed (Figure 4C and D). Similar to the mRNA results, 9 profiles were found in both groups, and the two most significant patterns (No. 22 and No. 5) included the largest number of differentially expressed genes (Table 1). In addition, the expression patterns of long non-coding RNAs included in No. 22 and No. 5 were similar to the mRNA data. These results indicated that liver tissues expressed different genes after PVL and that the largest number of genes was altered during the first 24 h after PVL.

Hypoxia, cell proliferation and apoptosis pathways enriched by GO

analysis

As different gene expression patterns were identified after PVL, we intended to investigate the biological pathways involved in this process. The genes in profiles No. 22 and No. 5 from the STC analysis, which were the most changed profiles, were uploaded to the DAVID online software to identify overrepresented GO categories. The results showed that genes in profile No. 22 from LLLs were significantly enriched in gene transcription, regulation and response to hypoxia for biological processes (BP); DNA and protein binding for molecular function (MF); and the cytoplasm and nucleus for cell components (CC) (Table 2). The genes in profile No. 5 from the LLLs were significantly enriched in BP, including cell cycle and regulation of apoptotic process as well as those presented in profile No. 22 (Table 2). For MF and CC, the enriched categories were same as those found in profile No. 22 (Table 2). The genes in profile No. 22 from the NLLs were enriched in BP, including regulation of cell proliferation, adhesion, migration and the apoptotic process; MF, including poly(A) RNA binding, ubiquitin-protein transferase activity, cadherin binding involved in cell-cell adhesion and p53 binding; and CC, including cytoplasm, nucleus, nucleoplasm, and Golgi apparatus (Table 3). The genes in profile No. 5 from the NLLs were enriched in BP, including DNA transcription, protein phosphorylation, cell proliferation and apoptosis; in MF, including protein, ATP, and DNA binding; and in CC, including cytoplasm extracellular exosome and membrane (Table 3). These results indicated that gene transcription and

protein activity were altered during the first 24 h after PVL in both LLLs and NLLs. During this period, the response to hypoxia was apparently activated in LLLs, and proliferation, adhesion and apoptosis of cells were affected in NLLs.

To gain better insight into the biological processes involved in the response to PVL, the differentially expressed genes at 24 h between LLLs or NLLs and the normal control liver were sent for GO BF analysis. A total of 1316 up-regulated and 1578 down-regulated genes were found in LLLs compared with those in the normal control tissues. The results of the GO analysis showed that the up-regulated genes were enriched in hypoxia response and the JNK and ERK signaling pathways (Figure 5A), indicating that PVL induced hypoxia reaction in LLLs. Negative regulation of cell proliferation and positive regulation of epithelial-to-mesenchymal transition (EMT) were also represented in the top rank (Figure 5A), suggesting that cell proliferation was inhibited and that EMT was activated. For down-regulated genes in LLLs, positive transcription regulation, protein phosphorylation and ubiquitin-dependent protein catabolic processes were overrepresented (Figure 5B), indicating that gene transcription and protein catabolic processes were inhibited in LLLs. For NLLs, 2151 genes were up-regulated, and 1605 genes were down-regulated. The up-regulated genes were significantly enriched in DNA replication and cell division, and the down-regulated genes were significantly enriched in organic cycling and protein phosphorylation (Figure 5C

and D), indicating hyperactivation of replication and cell division in NLLs.

Collectively, these data suggested that PVL induced the hypoxia response and inhibited gene transcription and protein catabolic processes in LLLs and activated DNA replication and cell division in NLLs.

Discussion

In the present study, we investigated gene expression in rat liver lobes after PVL using cDNA microarrays. Unique coding and non-coding gene expression profiles were found in both LLLs and NLLs. Moreover, we found that gene expression in LLLs and NLLs changed according to the time after PVL. Most of the gene expression changes occurred in the first 24 h after PVL, and these genes maintained their expression levels for 14 days after PVL. The GO analysis revealed that the genes differentially expressed in the first 24 h were mostly involved in cell proliferation and the cell cycle.

To the best of our knowledge, few studies have investigated gene expression profiles after PVL in a rat model by high-throughput technologies, such as cDNA microarrays. In the present study, hundreds of aberrantly expressed genes were found in LLLs and NLLs after PVL, providing evidence that these genes are involved in liver regeneration after PVL. The expression of genes involved in cell division and proliferation in NLLs was mostly altered by PVL, indicating that these were the main biological processes activated in NLLs and contributed to liver regeneration after PVL. These findings explain why Ki-67 staining increases after PVL/PVE have been reported by others

(Kusaka et al. 2006; Li et al. 2013). The enrichment of biological processes according to the GO analysis further supports the involvement of cytokines such as TGF- β in liver regeneration induced by PVL. TGF- β was reported to be related to hypertrophy in the non-embolized liver in patients (Kusaka et al. 2004). According to our data, TGF- β was included in the following GO terms: positive regulation of cell proliferation, heart development and positive regulation of cell migration. Additionally, genes involved in the JNK and ERK pathways were aberrantly expressed (Figure 5), supporting previous evidence that angiogenesis and insulin-like growth factor signaling contribute to liver regeneration (Rauchfuss et al. 2012). Moreover, hundreds of non-coding RNAs were aberrantly expressed, suggesting that epigenetic regulation is also involved in liver regeneration.

In agreement with previous reports, we found the highest NLLW/WLW ratio at 7 d after PVL in rats (Huisman et al. 2014; Liska et al. 2012). One week can be too long a time interval for some patients and limits the application of PVL in the clinic. We found that multiple pathways, such as positive regulation of cell proliferation and cell cycle, were activated in NLLW, suggesting that the signals involved in these pathways should be used with PVL to improve liver regeneration within a shorter time frame. It has been reported that HGF infusions significantly increased postoperative liver regeneration (Mangieri et al. 2016). Branched-chain amino acid supplementation was also reported to improve functional liver regeneration in patients undergoing PVE (Beppu et al.

2015). In addition, Schadde et al. reported that hypoxia is likely an accelerator of liver regeneration (Schadde et al. 2017). They found that compared with regenerating liver lobes from rats receiving PVL only or rats sham-operated at 3 and 12 hours, those from rats receiving PVL+ parenchymal transection showed significant hypoxia. In our study, the hypoxia response was activated in LLLs but not in NLLs at 24h after PVL. The parenchymal transection and short time lapse in Schadde's study may be the reason why we observed a different hypoxia status. Collectively, these findings suggest that modification of PVL to accelerate liver regeneration may be more efficient in clinical application.

Although we reported unique gene expression signatures of LLLs and NLLs after PVL, no functional assays were performed to verify the function of the specific coding and non-coding genes in atrophy or hypotrophy of liver lobes after PVL. In addition, several pathways involved in protein modification and lipid metabolism were also enriched in NLLs, indicating that regulation at the translational level also contributed to the biological response to PVL. A more thorough elucidation could be obtained if the present results are combined with the results of a proteomic analysis. Moreover, the functions of genes showing different expression profiles (No. 22 and No. 5) remain unclear and require further investigation.

Collectively, unique gene expression signatures were identified in LLLs and NLLs after PVL in rat model using cDNA microarrays. The results of

further analysis showed that both coding and non-coding genes were differentially expressed in LLLs and NLLs after PVL and that the expression levels changed over time. In addition, gene expression patterns assessed by STC showed that the largest number of genes was altered during the first 24 h at all three time points and that these expression levels were maintained for the following two weeks. The GO analysis showed that pathways involved in hypoxia, cell cycle and cell proliferation were activated in the liver lobes after PVL. Our findings provide a systemic view of the molecular alterations in liver lobes after PVL and provide insight into the mechanism underlying PVL-induced atrophy and hypertrophy.

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Figure legends

Figure 1. PVL induced atrophy in ligated liver lobes and hypertrophy in non-ligated liver lobes

- A) The whole liver weight (WLW)/ body weight (BW) ratio at different times (n=6) after PVL.
- B) The ligated liver lobe weight (LLLW)/BW and non-ligated liver lobes (NLLLW)/BW ratios at different times (n=6) after PVL.
- C) The LLLW/WLW and NLLLW/WLW ratios at different times (n=6) after PVL.

**** $P < 0.01$**

Figure 2. PVL altered gene expression in LLLs and NLLs at different time points after PVL

- A) Coding genes differentially expressed between LLLs and NLLs at the same time points (24 h, 7 d and 14 d) visualized by volcano plots based on the microarray data.
- B) Long non-coding genes differentially expressed between LLLs and NLLs at the same time points (24 h, 7 d and 14 d) visualized by volcano plots based on microarray data.
- C) Number of coding genes up-regulated or down-regulated between LLLs and NLLs at the time points 24 h, 7 d and 14 d after PVL.
- D) Number of non-coding genes up-regulated or down-regulated between LLLs and NLLs at the time points 24 h, 7 d and 14 d after PVL.

Figure 3. Genes were differentially expressed in LLLs or NLLs at different times after PVL

- A) Cluster analysis of coding gene expression in LLLs at different times (0 h, 24 h, 7 d and 14 d) after PVL
- B) Cluster analysis of coding gene expression in NLLs at different times (0 h, 24 h, 7 d and 14 d) after PVL
- C) Cluster analysis of non-coding gene expression in LLLs at different times (0 h, 24 h, 7 d and 14 d) after PVL
- D) Cluster analysis of non-coding gene expression in NLLs at different times (0 h, 24 h, 7 d and 14 d) after PVL

Figure 4. Dynamic coding and non-coding gene expression patterns in LLLs or NLLs according to the time after PVL

- A) The 11 expression profiles of coding genes that were statistically significant in the LLLs over time (0 h, 24 h, 7 d and 14 d).
- B) The 13 expression profiles of coding genes that were statistically significant in the NLLs over time (0 h, 24 h, 7 d and 14 d).

- C) The 9 expression profiles of non-coding genes that were statistically significant in the LLLs over time (0 h, 24 h, 7 d and 14 d).
- D) The 10 expression profiles of non-coding genes that were statistically significant in the NLLs over time (0 h, 24 h, 7 d and 14 d).

Figure 5. GO analysis results of differentially expressed genes between LLLs or NLLs and normal control liver at 24 h

- A) Top-ranking biological pathways enriched in up-regulated genes in LLLs compared to those in normal control livers according to the GO analysis.
- B) Top-ranking biological pathways enriched in down-regulated genes in LLLs compared to those in normal control livers according to the GO analysis.
- C) Top-ranking biological pathways enriched in up-regulated genes in NLLs compared to those in normal control livers according to the GO analysis.
- D) Top-ranking biological pathways enriched in down-regulated genes in NLLs compared to those in normal control livers according to the GO analysis.

94x204mm (300 x 300 DPI)

183x236mm (300 x 300 DPI)

148x188mm (300 x 300 DPI)

209x182mm (300 x 300 DPI)

187x154mm (300 x 300 DPI)

Table 1. Different Coding and non-coding gene expression patterns along with PVL time in LLL and NLLL

ligated liver lobes(LLL)			non-ligated liver lobes(NLLL)		
Profile NO.	Genes Counts	P value	Profile NO.	Genes Counts	P value
coding RNA					
Profile22	851	0	Profile22	875	0
Profile5	518	0	Profile5	688	0
Profile2	185	0	Profile16	253	0
Profile23	256	0	Profile25	168	0
Profile4	230	0	Profile26	87	0
Profile25	158	0	Profile19	540	0
Profile1	98	0	Profile23	187	0
Profile26	92	0	Profile4	177	0
Profile24	240	0	Profile2	115	0
Profile21	266	0	Profile8	569	0
Profile17	111	0.01	Profile20	294	0
			Profile17	101	0.01
			Profile1	56	0.03
non-coding RNA					
Profile22	664	0	Profile22	648	0
Profile5	527	0	Profile5	525	0
Profile4	250	0	Profile1	105	0
Profile23	195	0	Profile2	138	0
Profile1	77	0	Profile25	141	0
Profile2	120	0	Profile4	169	0
Profile25	87	0	Profile23	150	0
Profile26	47	0	Profile26	56	0
Profile20	147	0	Profile16	77	0.01
			Profile20	127	0.04

Table 2. GO analysis of genes in Profile 22 and Profile 5 from ligated liver lobes.

Profile 22				
Category	Term/gene function	Gene Count	%	P
GOTERM_BP_DIRECT	GO:0000122~negative regulation of transcription from RNA polymerase II promoter	38	5.9	0.01
	GO:0045892~negative regulation of transcription, DNA-templated	28	4.35	0.01
	GO:0006351~transcription, DNA-templated	37	5.75	0.02
	GO:0045944~positive regulation of transcription from RNA polymerase II promoter	45	6.99	0.03
	GO:0006357~regulation of transcription from RNA polymerase II promoter	23	3.57	0.03
	GO:0001666~response to hypoxia	16	2.48	0.03
	GO:0006355~regulation of transcription, DNA-templated	46	7.14	0.03
GOTERM_MF_DIRECT	GO:0005515~protein binding	77	11.96	0
	GO:0043565~sequence-specific DNA binding	30	4.66	0.01
	GO:0003682~chromatin binding	25	3.88	0.01
	GO:0003677~DNA binding	49	7.61	0.01
	GO:0044822~poly(A) RNA binding	51	7.92	0.01
	GO:0008270~zinc ion binding	48	7.45	0.01
	GO:0004672~protein kinase activity	16	2.48	0.03
GOTERM_CC_DIRECT	GO:0005737~cytoplasm	222	34.47	0
	GO:0005634~nucleus	204	31.68	0
	GO:0005794~Golgi apparatus	39	6.06	0.02
	GO:0043234~protein complex	33	5.12	0
	GO:0048471~perinuclear region of cytoplasm	33	5.12	0.01
	GO:0000790~nuclear chromatin	17	2.64	0
Profile 5				
Category	Term/gene function	Gene Count	%	P
GOTERM_BP_DIRECT	GO:0007049~cell cycle	12	1.86	0
	GO:0016310~phosphorylation	12	1.86	0.01
	GO:0000122~negative regulation of transcription from RNA polymerase II promoter	38	5.9	0.01
	GO:0001501~skeletal system development	10	1.55	0.01
	GO:0045892~negative regulation of transcription, DNA-templated	28	4.35	0.01

	GO:0006351~transcription, DNA-templated	37	5.75	0.02
	GO:0010033~response to organic substance	11	1.71	0.02
	GO:0045944~positive regulation of transcription from RNA polymerase II promoter	45	6.99	0.03
	GO:0006357~regulation of transcription from RNA polymerase II promoter	23	3.57	0.03
	GO:0001666~response to hypoxia	16	2.48	0.03
	GO:0006355~regulation of transcription, DNA-templated	46	7.14	0.03
	GO:0098609~cell-cell adhesion	13	2.02	0.04
	GO:0010468~regulation of gene expression	14	2.17	0.04
	GO:0042981~regulation of apoptotic process	11	1.71	0.05
GOTERM_MF_DIRECT	GO:0005515~protein binding	77	11.96	0
	GO:0043565~sequence-specific DNA binding	30	4.66	0.01
	GO:0003682~chromatin binding	25	3.88	0.01
	GO:0003677~DNA binding	49	7.61	0.01
	GO:0044822~poly(A) RNA binding	51	7.92	0.01
	GO:0008270~zinc ion binding	48	7.45	0.01
	GO:0003700~transcription factor activity, sequence-specific DNA binding	35	5.43	0.03
	GO:0004672~protein kinase activity	16	2.48	0.03
GOTERM_CC_DIRECT	GO:0005737~cytoplasm	222	34.47	0
	GO:0005634~nucleus	204	31.68	0
	GO:0000790~nuclear chromatin	17	2.64	0
	GO:0005654~nucleoplasm	78	12.11	0
	GO:0005730~nucleolus	42	6.52	0
	GO:0043234~protein complex	33	5.12	0
	GO:0048471~perinuclear region of cytoplasm	33	5.12	0.01
	GO:0005794~Golgi apparatus	39	6.06	0.02

Table 3. GO analysis of genes in Profile 22 and Profile 5 from non-ligated liver lobes.

Profile 22				
Category	Term	Count	%	P
GOTERM_BP_DIRECT	GO:0008284~positive regulation of cell proliferation	27	0.03	0.02
	GO:0007507~heart development	18	0.02	0.02
	GO:0030335~positive regulation of cell migration	16	0.02	0
	GO:0098609~cell-cell adhesion	14	0.01	0.03
	GO:0007049~cell cycle	13	0.01	0
	GO:0007605~sensory perception of sound	13	0.01	0
	GO:0042981~regulation of apoptotic process	13	0.01	0.01
	GO:0016192~vesicle-mediated transport	11	0.01	0.01
GOTERM_MF_DIRECT	GO:0005515~protein binding	79	11.92	0
	GO:0044822~poly(A) RNA binding	60	9.05	0
	GO:0046982~protein heterodimerization activity	28	4.22	0.03
	GO:0032403~protein complex binding	24	3.62	0
	GO:0003723~RNA binding	24	3.62	0.04
	GO:0004842~ubiquitin-protein transferase activity	19	2.87	0.02
	GO:0019904~protein domain specific binding	18	2.71	0.03
	GO:0008022~protein C-terminus binding	14	2.11	0.02
	GO:0098641~cadherin binding involved in cell-cell adhesion	14	2.11	0.03
	GO:0005179~hormone activity	12	1.81	0
	GO:0005516~calmodulin binding	11	1.66	0.04
	GO:0044325~ion channel binding	10	1.51	0.03
	GO:0019903~protein phosphatase binding	8	1.21	0.03
	GO:0002039~p53 binding	7	1.06	0.03
	GO:0000980~RNA polymerase II distal enhancer sequence-specific DNA binding	7	1.06	0.03
GOTERM_CC_DIRECT	GO:0005737~cytoplasm	209	0.21	0
	GO:0005634~nucleus	198	0.19	0
	GO:0005654~nucleoplasm	76	0.07	0.01
	GO:0005794~Golgi apparatus	49	0.05	0
	GO:0005730~nucleolus	47	0.05	0
	GO:0005622~intracellular	47	0.05	0.02
	GO:0005887~integral component of plasma membrane	43	0.04	0.03
	GO:0043234~protein complex	38	0.04	0
	GO:0048471~perinuclear region of cytoplasm	38	0.04	0

	GO:0005783~endoplasmic reticulum	38	0.04	0.03
Profile 5				
Category	Term	Count	%	P
GOTERM_BP_DIRECT	GO:0045944~positive regulation of transcription from RNA polymerase II promoter	49	9.18	0
	GO:0006355~regulation of transcription, DNA-templated	43	8.05	0.01
	GO:0045893~positive regulation of transcription, DNA-templated	36	6.74	0
	GO:0006351~transcription, DNA-templated	34	6.37	0.01
	GO:0000122~negative regulation of transcription from RNA polymerase II promoter	33	6.18	0.01
	GO:0006468~protein phosphorylation	27	5.06	0
	GO:0045892~negative regulation of transcription, DNA-templated	27	5.06	0
	GO:0007165~signal transduction	27	5.06	0.02
	GO:0055114~oxidation-reduction process	27	5.06	0.03
	GO:0043066~negative regulation of apoptotic process	24	4.49	0.01
	GO:0008285~negative regulation of cell proliferation	23	4.31	0
	GO:0014070~response to organic cyclic compound	16	3	0.01
	GO:0007623~circadian rhythm	15	2.81	0
	GO:0055085~transmembrane transport	15	2.81	0
	GO:0001701~in utero embryonic development	15	2.81	0.02
	GO:0010468~regulation of gene expression	14	2.62	0.01
	GO:0001934~positive regulation of protein phosphorylation	13	2.43	0
	GO:0030335~positive regulation of cell migration	12	2.25	0.02
	GO:0030182~neuron differentiation	11	2.06	0.01
	GO:0046777~protein autophosphorylation	11	2.06	0.03
	GO:0030324~lung development	10	1.87	0.01
	GO:0043524~negative regulation of neuron apoptotic process	10	1.87	0.03
	GO:0048146~positive regulation of fibroblast proliferation	9	1.69	0
GOTERM_MF_DIRECT	GO:0005515~protein binding	70	13.11	0
	GO:0005524~ATP binding	52	9.74	0.02
	GO:0008270~zinc ion binding	47	8.8	0
	GO:0003677~DNA binding	46	8.61	0
	GO:0003700~transcription factor activity, sequence-specific DNA binding	35	6.55	0
	GO:0042803~protein homodimerization activity	33	6.18	0.02

	GO:0043565~sequence-specific DNA binding	32	5.99	0
	GO:0046982~protein heterodimerization activity	25	4.68	0.02
	GO:0032403~protein complex binding	24	4.49	0
	GO:0003682~chromatin binding	23	4.31	0.01
GOTERM_CC_DIRECT	GO:0005737~cytoplasm	163	30.52	0.01
	GO:0070062~extracellular exosome	99	18.54	0
	GO:0016020~membrane	85	15.92	0
	GO:0005829~cytosol	70	13.11	0
	GO:0043234~protein complex	31	5.81	0
	GO:0048471~perinuclear region of cytoplasm	31	5.81	0
	GO:0043025~neuronal cell body	29	5.43	0
	GO:0009986~cell surface	28	5.24	0.01
	GO:0043231~intracellular membrane-bounded organelle	28	5.24	0.03
	GO:0030054~cell junction	22	4.12	0

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