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Enhanced liver regeneration following changes induced by hepatocyte-specific genetic ablation of integrin-linked kinase

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Abstract

Following liver regeneration after partial hepatectomy, liver grows back precisely to its original mass and does not exceed it. The mechanism regulating this “hepatostat” is not clear and no exceptions have been found to date. While pathways initiating liver regeneration have been well studied, mechanisms involved in termination of liver regeneration are unclear. Here we report that Integrin Linked Kinase (ILK) (involved in transmission of the extracellular matrix (ECM) signaling via integrin receptors) and/or hepatic adaptations that ensue following ILK hepatocyte-targeted removal, are critical for proper termination of liver regeneration. Following partial hepatectomy (PHX), mice with a liver-specific ILK ablation (ILK-KO-Liver) demonstrate a termination defect resulting in 58% larger liver than their original pre-PHX mass. This increase in post-PHX liver mass is due to sustained cell proliferation driven in part by increased signaling through HGF/Met, and β -catenin pathway and Hippo Kinase pathways. The data indicate that ECM-mediated signaling via ILK is essential in proper termination of liver regeneration. This is the first evidence of a defect leading to impaired termination of regeneration and excessive accumulation of liver weight following partial hepatectomy.

Keywords

Extracellular matrix; integrin linked kinase; termination of regeneration

Liver is known for its remarkable capacity to regenerate following 2/3 partial hepatectomy (PHx) or injury (1-3). During regeneration, various cell types in the liver undergo controlled proliferation resulting in the growth of remnant liver lobes back to precisely the pre-PHx liver mass. The entire process of liver regeneration following PHx takes up to 5 to 7 days in rodents during which the remnant liver lobes grow through extensive proliferation of all hepatic cell types. When the pre-PHx liver size is achieved, liver growth and cell proliferation stops. The roles of various growth factors, cytokines and nuclear receptors in initiation of liver regeneration have been extensively studied (1-4). On the contrary, the mechanisms involved in termination of liver regeneration and in precise regulation of the liver size following PHx are not as well understood. Molecules such as TGF β , Activin-A, and IL-1 β may be involved in termination of regeneration but the precise mechanisms underlying their action remain elusive (5,6). Following liver regeneration, liver grows back precisely to its original mass and does not exceed it (2). Hepatic transplantation models have also been used to show that livers from small animals transplanted to animals of larger size

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grow I size until the “correct” size is attained (7). The mechanism regulating this “hepatostat” is not clear and no exceptions have been found to date. We have recently shown that hepatocyte-targeted genetic ablation of Integrin Linked Kinase (ILK), a protein involved in transmitting extracellular matrix (ECM) signals via integrins, changes the proliferation kinetics of hepatocytes in normal livers and results in an adapted liver status in which there is a new set of integrins, modified extracellular matrix and a final liver whose size is larger than the normal (8). There are also multiple observations that ECM in hepatocyte cultures suppresses cell proliferation and induces differentiation (9-12). The above findings underscore the key importance of ECM in regulating differentiation and proliferation of hepatocytes. Our recent findings with ILK hepatocyte-targeted removal suggest that the adaptations resulting from ILK elimination affect the “hepatostat” and result in a larger liver size (8). In view of the above findings on ILK and ECM, we hypothesized that either ILK itself or the adaptations resulting from its removal from hepatocytes may also affect termination of liver regeneration. Here we report that following partial hepatectomy (PHx), mice with a liver-specific ILK ablation (ILK-KO-Liver) and at 30 weeks after birth demonstrate a termination defect resulting in a liver 58% larger than the original pre-PHx mass (starting from 2X above control and ending at 3.2X above control mouse liver weight). This increase in post-PHx liver mass is due to sustained cell proliferation driven in part by increased signaling through HGF/Met, β -catenin and Hippo Kinase pathways. This is the first evidence of a defect leading to impaired termination of regeneration and excessive accumulation of liver weight following partial hepatectomy.

Materials and Methods

Antibodies

The following primary antibodies were used in this study: Rabbit anti-ILK, Rabbit anti-YAP, Rabbit anti-phosphorylated YAP, rabbit anti-phosphorylated Met, mouse anti p42/p44 MAPK, mouse anti-phosphorylated p42/p44, rabbit anti-p38 MAPK, rabbit anti-P27, rabbit anti-phosphorylated p38 MAPK, rabbit anti-Tyr654-phosphorylated β -catenin (1:1000 dilution, Cell Signaling Technologies, Danvers, MA); mouse anti-c-Myc, mouse anti-cyclin D1, rabbit anti-TGF β 1, Mouse anti-Met, mouse anti-E-cadherin (1:200 dilution, Santa Cruz biotechnology, Santa Cruz, CA), goat anti-HGF, mouse anti-PCNA (Dako, Carpinteria, CA), mouse anti-TCF-4 (1:200 dilution, Upstate Technologies, Lake Placid, NY) and mouse anti- β -actin (1:5000 dilution, Chemicon, Temecula, CA). Goat anti-mouse, donkey anti-goat and donkey anti-rabbit secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA) and were used at 1:50,000 dilution.

Animals

The ILK-floxed animals used in this study were generated as described previously(1) and were mated with mice expressing Cre-recombinase under the control of hepatocyte-specific promoters α -fetoprotein enhancer-albumin promoter. All the Cre-expressing animals were always maintained in a heterozygous state to avoid any Cre-induced toxicity. Genotyping was performed by PCR from DNA isolated from the mouse tails using previously published ILK primer sequences(1). For the genotyping of the Cre transgenic animals we used the following set of primers: 5'-GCG GTC TGG CAG TAA AAA CTA TC-3' (forward) and 5'-GTG AAA CAG CAT TGC TGT CAC TT-3'. All animals were housed in the animal facility of the University of Pittsburgh and all experiments were performed under protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pittsburgh. Animals were treated humanely according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health.

Partial hepatectomy (PHx)

AFP-albumin-ILK-KO animals of 25-30 weeks of age along with their WT-Cre negative siblings were subjected to a 35-40% partial hepatectomy (PHx). The procedure was performed as described previously(1,2). Mice were sacrificed at 2, 3 5, 7 and 14 days post PHx and the weight of their livers as well as the whole body was obtained. The ratio of the weight of the remaining liver after PHx over the weight of the whole animal was taken as the liver to body weight ratio. The results obtained were the mean of three different animals per time point and condition. Statistical analysis was performed using the Excel Software (Microsoft Office) and a p-value of less than 0.05 was considered statistically significant.

Protein isolation and Western Blotting

Total protein was isolated from the mouse hepatocytes, non-parenchymal cells of the liver or whole livers from the AFP-albumin-ILK-KO, albumin-ILK-KO or Foxa3-ILK- KO animals using 1% sodium dodecyl sulfate (SDS) in RIPA buffer (20 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5% NP-40, 1% TX-100, 0.25% Sodium Deoxycholate (DOC), 0.6- 2 µg/ml aprotinin, 10µM Leupeptin, 1µM Pepstatin). Protein concentrations of all lysates were determined using the bicinchoninic acid protein assay reagents (BCA method) (Pierce Chemical Co., Rockford, IL). Nuclear proteins were prepared using the NE-PER nuclear and cytoplasmic protein isolation kit (Pierce, Rockford IL) according to the manufacturer's protocol.

Total cell lysates made in Ripa buffer (50 µg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis in 4% to 12% NuPage Bis-Tris gels with MOPS buffer (Invitrogen, Carlsbad, CA), then transferred to Immobilon-P membranes (Millipore, Bedford,MA) in NuPAGE transfer buffer containing 20% methanol. Membranes were stained with Ponceau S to verify loading and transfer efficiency. Membranes were probed with primary and secondary antibodies in Tris-buffered saline Tween 20 containing 5% nonfat milk, then processed with SuperSignal West Pico chemiluminescence substrate (Pierce, Rockford, IL) and exposed to a X-ray film (Lab Product Sales, Rochester, NY).

Immunohistochemistry

Paraffin-embedded liver sections (4 µm thick) were used for immunohistochemical staining. Antigen retrieval was achieved by heating the slides in the microwave at high power in citrate buffer for 10 minutes. The tissue sections were blocked in blue blocker for 20 minutes followed by incubation with pertinent primary antibody overnight at 4°C. The primary antibody was then linked to biotinylated secondary antibody followed by routine avidin-biotin complex method. Diaminobenzidine was used as the chromogen, which resulted in a brown reaction product.

Microarray analysis

cRNA preparation—Total RNA was extracted and purified with Qiagen RNeasy kit (Qiagen, San Diego, CA) from whole livers harvested from ILK-KO and WT at various time points after PHX. Five micrograms of total RNA were used in the first strand cDNA synthesis with T7-d(T)24 primer (GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG-(dT)24) by Superscript™ II (GIBCO-BRL, Rockville, MD). The second strand cDNA synthesis was carried out at 16°C by adding E. coli DNA ligase, E. coli DNA polymerase I and RnaseH in the reaction. This was followed by the addition of T4 DNA polymerase to blunt the ends of newly synthesized cDNA. The cDNA was purified through phenol/chloroform and ethanol precipitation. The purified cDNA were then incubated at 37°C for 4 hours in an *in vitro* transcription reaction to produce cRNA labeled with biotin using MEGAscript™ system (Ambion, Inc, Austin, TX).

Affymetrix oligo-CDNA array hybridization—Fifteen to 20 µg of cRNA were fragmented by incubation in a buffer containing 200 mM Tris-acetate, pH 8.1, 500 mM KOAc, 150 mM MgOAc at 95°C for 35 minutes. The fragmented cRNA were then hybridized with a pre-equilibrated Affymetrix chip (R430 2.0) at 45°C for 14–16 hours. After the hybridization cocktails were removed, the chips were then washed in a fluidic station with low-stringency buffer (6x SSPE, 0.01% Tween 20, 0.005% antifoam) for 10 cycles (2 mixes/cycle) and stringent buffer (100 mM MES, 0.1 M NaCl and 0.01% Tween 20) for 4 cycles (15 mixes/cycle), and stained with SAPE (Strepto-avidin Phycoerythrin). This was followed by incubation with biotinylated mouse anti-avidin antibody, and restained with SAPE. The chips were scanned in a HP ChipScanner (Affymetrix Inc, Santa Clara, CA) to detect hybridization signals. For quality assurance, all samples were run on Affymetrix test-3 chips to evaluate the integrity of RNA. Samples with RNA 3'/5' ratios less than 2.5 were accepted for further analysis.

Results

The adaptations and changes in the livers of ILK-Liver-KO- mice have been previously described (8). Partial hepatectomies were performed in 30 week old mice with hepatocyte-targeted ILK removal, using Cre expressing mice of the same age as control. Changes in liver weights and proliferation kinetics are shown in Table 1, Fig. 1 and Figure 2 (A and B). In Table 1, the data show the liver weights (in grams) prior to and after partial hepatectomy performed in control and ILK-KO-Liver mice. The same data are plotted in Figure 1 as percent of liver weight regained after the operation. Both Table 1 and Figure 1 show that whereas in control mice liver weight returns to the original size prior to hepatectomy, in ILK-KO-Liver mice liver weight surpasses (at Day 7) that of the pre-hepatectomy size and it continues to grow beyond that. At day 14 after PHx, a time in which according to all literature liver regeneration has terminated, the liver weight of the ILK-KO-Liver mice surpasses that of the pre-hepatectomy level by 58%–58%. The control mice exhibited the typical pattern of termination of liver regeneration with liver weights returning to their pre-PHx weights (1.36 ± 0.2 grams) by 14 days after PHx.

The detail proliferation kinetics of hepatocytes at different times after PHx are shown in Figure 2A. In both types of mice, there is a typical initial increase (2 and 3 days post PHx) and later decrease (5, 7 and 14 days post-PHx) in cell proliferation, as assessed by proliferating cell nuclear antigen (PCNA) immunohistochemistry. Compared to Control mice, the ILK-KO-Liver mice exhibited a slightly slower increase in initial cell proliferation at 2 days post-PHx, but had sustained cell proliferation at 3, 5, 7 and 14 days post-PHx (Fig 2A). The main difference in liver histology between the two types of mice was the persistent hepatocyte proliferation of hepatocytes in the ILK-KO-Liver mice at 5, 7 and 14 days after PHx (Figure 2B).

A detailed microarray analysis (Affymetrix) was performed to investigate mechanisms involved in the termination defect in the ILK-KO-Liver mice. No major difference in gene expression of potential extracellular signals (2) was seen with the exception of hepatocyte growth factor (HGF). HGF gene expression was 5-fold higher before PHx in the ILK-KO-Liver mice and remained higher at 7 and 14 days post-PHx (Figure 3A and 3B). There was increased protein for both HGF and its receptor Met for Days 7 and 10 in the ILK-KO-Liver mice, whereas a decline for the same time points was seen in the Control mice (Fig. 3B). We have shown in previous studies (13,14) that beta catenin migrates to hepatocyte nuclei from the earliest stages after PHx. The data in Figure 3C show that there is increased presence of beta catenin in nuclei in the ILK-KO-Liver mice. We also investigated AKT/Protein Kinase B, a signaling kinase linked to both ILK and HGF (15). ILK-KO-Liver mice had substantially higher total as well as phosphorylated AKT protein especially at Days 7–14

(Fig. 4A). We further investigated ERK1/2 (p42/p44 MAPK) and p38 MAPK, the two main downstream mitogen-activated kinase pathways activated by HGF. Previous studies have reported the role of p42/p44 MAPK in activation and p38 MAPK in inhibition of cell proliferation (16,17). There was no difference in the total protein of p42/p44 MAPK (data not shown) or p38MAPK. Differences were noticed however in phospho-p38 MAPK (Fig. 3B). We observed an increase in phosphorylation (activation) of p38 MAPK in the ILK-KO-Liver liver at 2, 3 and 5 days after PHx, which decreased at 7 and 14 days post PHx. This is consistent with the lower cell proliferation observed in the ILK-KO-Liver mice at 2 days post-PHx and later sustained proliferation at 7 and 14 days after PHx. Previously, it has been demonstrated that P38 MAPK can inhibit cell cycle progression during liver regeneration (18).

To further analyze signaling pathways during the time of prolonged proliferation in the KO mice (days 7-14 after PHx), we investigated levels of cell cycle genes. We observed overall increased and sustained expression of c-Myc in the ILK-KO-Liver mice over the 14 day post- PHx time course. Additionally, expression of the CDK2 inhibitor p27, a cell cycle inhibitor, was consistently lower in ILK-KO-Liver mice at 5, 7 and 14 days post-PHx. (Fig. 3C). TGF β is another molecule implicated in termination of liver regeneration due to its anti-proliferative activity (19). However, no significant changes in TGF β 1 protein levels were seen between the two groups (Fig. 4D).

Recently the role of Hippo Kinase pathway in regulation of organ size in *Drosophila* as well as of liver in mammals has been reported (20). The mammalian Hippo Kinase pathway converges on yes-associated protein (YAP), which plays a role in liver size regulation and cancer development (20). We investigated whether ILK loss causes changes in YAP expression during liver regeneration. Western blot analysis for YAP indicated substantially higher YAP protein in ILK-KO-Liver livers at 5, 7 and 14 days after PHx (Fig. 4E). Phosphorylation of YAP results in its nuclear export and degradation, which leads to decrease in cell proliferation. The ILK-KO-Liver mice had overall lower phosphorylated YAP protein at all time points following PHx as compared to the Control mice. Furthermore, immunohistochemistry for YAP indicated higher cytoplasmic and nuclear YAP in ILK-KO-Liver mice with loss of the restriction of positive immunohistochemistry to periportal regions observed in the Control mice (Fig. 4E). These data indicate that ILK signaling contributes to control of the Hippo kinase pathway, and particularly YAP activation in the liver.

To understand the whether the termination defect in ILK-KO-Liver mice is coupled with defective hepatic differentiation, a comprehensive microarray analysis was conducted using liver mRNA collected at 0, 2, 3, 5, 7 and 14 days after PHx. The microarray study revealed that in addition to excessive liver weigh, ILK-KO-Liver mice also have problems in regulating the differentiation status of the liver at the end of regeneration. We compared the expression of the 120 most expressed genes at time 0 (day of hepatectomy) in each of the two categories of mice and followed their expression levels by Affymetrix gene arrays at different days after PHx (Fig. 5). In Control mice, levels of expression of the top most expressed genes returned mostly to pre-PHx values at Day 14 after PHx. In ILK-KO-Liver mice on the other hand, there was a wide increase in the expression of many of the same top 120 genes and several of them remained elevated at Day 14. Selenoprotein P was the most elevated gene for the ILK-KO-Liver mice at Day 14 after PHx. Major Urinary Protein 1 became elevated in Control mice at Day 5 but returned to normal levels by Day 14. It did remain elevated in the ILK-KO-Liver mice. These data indicate that targeted ablation of ILK and the associated secondary changes result in defective control of hepatocyte differentiation and block return of gene expression to pre-PHx levels, in addition to inappropriate termination of growth and excess liver weight accumulation.

Discussion

The data in this manuscript add another dimension to the consequences of hepatocyte-specific elimination of ILK and highlight the complex relationship between liver and its ECM. Overall, there is not much ECM in the liver lobule that is visible by light microscopy. Biochemical studies however demonstrate a wide variety of ECM proteins in the pericellular space surrounding hepatocytes, including syndecan, glypican 3, decorin, laminin, different types of collagen, etc. This material is partially degraded and remodeled during the early stages of liver regeneration and it is re-synthesized towards the end of the regenerative process (1,2). Artificial degradation of this matter by perfusion of the liver with collagenase makes hepatocytes responsive to HGF and, after isolation from the organ, renders them into the cell cycle (21). In the absence of complex matrix material, hepatocytes in culture proliferate in response to HGF or EGF but they lose most of the hepatocyte-associated gene expression patterns (11). Addition of complex ECM material to hepatocyte cultures (e.g. Matrigel, collagen type I gels) inhibits hepatocyte proliferation and restores their differentiation (11,22).

Our studies with liver-targeted removal of ILK have aimed to determine whether the strong effects of ECM on hepatocytes actually operate *in vivo*, in the context of the whole organ. Since it is practically impossible to eliminate ECM from an intact organ, elimination of the proteins responsible for transmission of the ECM signals to hepatocytes became a feasible alternative, when ILK^{loxP/loxP} mice became available. Integrin signaling involves multiple components and interactions with other receptors, etc. There are two proteins however primarily involved with transmission of the integrin signal, FAK (focal adhesion kinase) and ILK (23). Liver targeted elimination of ILK disrupts in part the integrin signal. We have seen so far two consequences of this ablation.

- a. Acute elimination of ILK from the liver. This was accomplished by injecting adenoviral constructs expressing Cre recombinase into the peripheral branches of the portal vein of ILK^{loxP/loxP} mice. Acute removal of ILK, presumably by interfering with integrin signaling but also perhaps through other mechanisms, led to massive hepatocyte apoptosis, fulminant hepatitis and death of the mice (24). This is consistent with the known activity of ILK to suppress anoikis (25).
- b. Genetic ablation of ILK targeted to hepatocytes. Contrary to the acute removal, genetic elimination of ILK (ILK^{loxP/loxP} mice mated with mice expressing Cre under the Afp enhancer/Albumin promoter) was tolerated during embryonic development. There were subsequent changes, however, in liver structure and there was increase in hepatocyte proliferation, increased deposition of new type extracellular matrix, appearance of new integrins and enlargement of liver (for details, please reference (8)). At 30 weeks after birth, most of these phenomena become quiescent. These were the mice used to derive the data described in this manuscript.

Given the multiplicity of changes accompanying removal of ILK, it is not easy to assign the defect in termination of regeneration to any specific single signaling system. The cybernetic interconnections between the different signaling systems are quite complex. It is reasonable to postulate, however, that the “over-regeneration” seen in these mice is a consequence of the adaptations resulting from one central event, the disruption or alteration of the signaling of ECM to hepatocytes because of the removal of ILK.

Given the dual impact of ECM in culture towards both proliferation and differentiation of hepatocytes, it was interesting to see in our recent work (8) that immediately following removal of ILK and as proliferation of hepatocytes was increasing, there was a decrease in hepatocyte differentiation (fig. 6 and Table 1 of reference (8)). This was comparable to what

was seen when hepatocytes are placed in primary culture, in the absence of matrix. This dual impact on both proliferation and hepatocyte differentiation is seen again in this study, from the results of Figure 5. Whereas gene expression (top 100 expressed genes, Table 1 reference (8) returns to pre-hepatectomy levels at the end of regeneration, the expression of the same genes does not return to normal at Day 14 in the ILK-KO-Liver mice. Many of the genes stay on at a high level.

It is not clear whether the abnormalities related to termination of regeneration, maintenance of the proper liver to body weight ratio and setting the right level of expression of hepatocyte associated genes can all be accounted primarily by ILK removal or by the secondary consequences of its removal seen over the entire liver. We believe however, that studies involving mice with specific genetic ablations should be probed for such changes. Our studies underscore that despite the apparent “normality” of the livers of ILK-KO-Liver mice (other than the increase in weight), there are many irregularities that would not be detected other than by comprehensive analysis of gene expression.

Our findings underscore the need to understand the complex signaling between hepatocytes and ECM in vivo. Alterations in matrix composition and deposition are one of the most frequent events in the histopathology of liver disease. Our findings suggest that ECM changes seen in such conditions, e.g. in micronodular cirrhosis regardless of etiology, may have defining and under-appreciated role to play for the performance (differentiation and proliferation) of the hepatocytes and consequently of the whole liver.

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ABBREVIATIONS

ECM	Extracellular Matrix
EGF	Epidermal Growth Factor

FAK	Focal Adhesion Kinase
HGF	Hepatocyte Growth Factor
ILK	Integrin Linked Kinase
KO	Knock-out
PHx	Partial hepatectomy
PCNA	Proliferating cell Nuclear Antigen
TGFβ	Transforming Growth Factor beta

Figure 1.

The data show the percentile of liver weight regained after partial hepatectomy in the control and ILK-KO-Liver mice. Following partial hepatectomy, the liver weights of Control and ILK-KO-Liver mice drop down to 54% and 53% of the original, respectively. At Day 14 after PHx, the liver weight of the control mice reaches 100% of the original whereas that of ILK-KO-Liver mice reaches 158% of the pre-hepatectomy weight. The data show the mean of at least 3 animals per point and are entirely derived from the data of Table 1. Statistical significance of differences is also shown in Table 1.

Fig. 2.

A. Percent of PCNA positive cells in the livers of ILK-KO-Liver and Control mice at various time points after PHx. ILK-KO-Liver mice have sustained excess cell proliferation at 5, 7 and 14 days post-PHx. The data show the mean and standard error from observations from three separate animals. The asterisks (*) indicate differences that are statistically significant at $p < 0.05$. (B), Representative photomicrographs of PCNA immunohistochemistry in Control mice and ILK-KO-Liver mice at 14 days after PHx demonstrate prolonged proliferation of hepatocytes in livers of ILK-KO-Liver mice.

Figure 3.

Changes in HGF and its receptor cMet and levels of total and nuclear beta catenin after partial hepatectomy. In A, data indicated expression values of HGF RNA in pooled samples of three animals per point. (CO: Control mice). B and C show representative western blots of HGF, Met and beta catenin (total (whole cell) and nuclear) from at least three experiments. There is increase in cMet at days 7 and 14 and increase in nuclear beta catenin at Days 2-7 post PHx in livers of ILK-KO-Liver mice compared to Control (CO).

Figure 4.

Changes in cell cycle related proteins in ILK-KO-Liver mice after PHx, compared to Control. A and B: Western blots of total and phospho- AKT and total and phospho-p38 MAPK at different times after PHx. (C) Western blot analysis of c-myc and p-27 showing higher levels of c-myc and lower levels of p27 in ILK-KO-Liver mice. (D) Western blot showing no significant differences in TGF β 1 protein at 5, 7 and 14 days after PHx between ILK-KO-Liver and Control mice. (E) Western blot analysis of total YAP protein and phosphorylated YAP protein in ILK-KO-Liver and Control mice after PHx. At days 5, 7 and 14 after PHx there is an increase in total YAP and a decrease in the phospho-YAP to total YAP ratio in the ILK-KO-Liver mice. In D, representative photomicrographs of immunohistochemistry for YAP in Control and ILK-KO-Liver mice at 14 days post-PHx indicating higher levels of cytoplasmic and nuclear YAP protein in ILK-KO-Liver mice. Data expressed as western blots are representative of at least three separate experiments.

Figure 5.

The top 120 expressed genes in time 0 (pre-PHx) in ILK-KO mice were identified by ranking of expression values in the Affymetrix gene array results. (for details, please supplement of reference (8)). The figure shows the expression of these genes at different times after PHx in both Control and ILK-KO-Liver mice. Gene expression values were temporarily increased for some of the genes, but they all returned close to pre-hepatectomy values for Control mice. The expression values for ILK-KO-Liver mice however increased and for most of the genes remained elevated at Day 14 after PHx. Selenoprotein P had the most elevated expression in ILK-KO-Liver. MUP1 and Hemopexin (the highest expressions in Control at Day 7) also remained elevated at Day 14 in the ILK-KO-Liver group.

TABLE 1

Liver weights of Control and ILK-KO-Liver mice at partial hepatectomy (PHx) and different times thereafter.

Liver Weight (grams)	Control	ILK-KO-Liver
<u>Prior to PHx</u>	1.36 ± 0.03	1.67 ± 0.12
<u>Immediately after PHx</u>	0.6 ± 0.17	0.9 ± 0.20
3 Days	0.69 ± 0.09	1.09 ± 0.09
5 Days	0.95 ± 0.12	1.82 ± 0.23
7 Days	1.18 ± 0.08	1.71 ± 0.12
14 Days	1.42 ± 0.10	2.64 ± 0.12

The data show the liver weights in terms of number of grams (mean± standard error) from at least three separate animals per time point. The liver weights of mice prior to hepatectomy were obtained by sacrificing animals specifically for that purpose. The results show the gradual increase of liver weight in each animal type. In control mice, the difference between liver weight prior to hepatectomy and at 14 days after hepatectomy is not statistically significant. The same difference for ILK-KO-Liver mice is statistically significant at $p < 0.005$.